MYCOLOGIA

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MYCOLOGIA

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VOL. LI JANUARY–FEBRUARY, 1959 NO. 1

A MONOCYSTIC GENUS OF THE MYXOBACTERALES (SCHIZOMYCETES)[1]

JOHN E. PETERSON[2]

Roland Thaxter described a myxobacterial species in 1893 which he named *Polyangium simplex* because of its solitary-cyst fruiting habit. Krzemieniewska and Krzemieniewski (**1926**) described a second species of solitary-cysted myxobacterium, *Polyangium ochraceum*, which they isolated from Polish soils. Neither of the species had been reported by others than the original describers until the writer found a myxobacterium on the bark of living trees that he determined as Thaxter's *Polyangium simplex*. The writer also has found two other myxobacteria on the bark of living trees that he has concluded are undescribed species, both of which produce single-cysted fruiting bodies.

Four species of myxobacteria now have been found that produce fruiting bodies consisting of solitary, definite-walled cysts. It is incongruous to continue to designate such organisms as members of the genus *Polyangium*, which means "many vessels," when these four species produce single-cysted fruiting bodies. Therefore a new genus, *Haploangium*, of the family Polyangiaceae is proposed to encompass the four species of myxobacteria characterized by the production of fruiting bodies consisting of solitary cysts.

[1] A portion of a thesis presented to the School for Advanced Graduate Studies of Michigan State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Haploangium gen. nov.

(Ethymology: Greek *haplo,* single; Greek *angion,* vessel.)

Fruiting bodies consisting of a single, solitary cyst, sessile on the substrate. Cyst wall well-developed, persistent and distinct, formed of dried slime; imbedded or not in further slime layers.

TYPE SPECIES: *Myxobacter simplex* Thaxter.

The genus *Haploangium,* as proposed in this paper, contains four species, *H. simplex* (Thaxter), *H. ochraceum* (Krzemieniewska & Krzemieniewski), *H. minor,* sp. nov., and *H. rugiseptum* sp. nov. *H. simplex* has been found on the bark of three different species of living trees by the writer, the first reported occurrence of the species since Thaxter described it on decaying moist wood and bark in 1893. The writer has also studied some of Thaxter's material, which was graciously lent to him by Dr. I. Mackenzie Lamb of the Farlow Herbarium, Harvard University.

H. ochraceum has been recorded only by the Krzemieniewskys (**1926**). No specimens are available and the writer has never observed this species. Of course, both of the species of *Haploangium* described in this paper for the first time have been studied only by the writer. All four of the species of the genus *Haploangium* have been reported on a minimum of occasions and by a minimum of observers. Only three workers have contributed to the genus: Thaxter (**1893**) has described one species, the Krzemieniewskys (**1926**) have described one species, and the writer is describing two species and also has studied Thaxter's *Polyangium simplex.*

Haploangium simplex (Thaxter) comb. nov.

Myxobacter simplex Thaxter, Bot. Gaz. **18**: 29. 1893; *Polyangium simplex* Thaxter, Bot. Gaz. **37**: 414. 1904.

Fruiting bodies solitary cysts, irregularly rounded or oval, dull red-orange to red-yellow, becoming bright yellow with age, with glistening spots of irridescence on the surface, ranging in size from 55 × 90 μ for those on bark to 350–400 μ for those described by Thaxter. In transmitted light, cyst wall yellow and definite. Some cysts surrounded by a yellow slime envelope in which many entrapped rods can be seen, but other cysts without such envelope. Spore mass clearly seen through the walls of those cysts possessing a slime envelope, flesh-colored; walls of those cysts not possessing slime envelopes thicker and more deeply pigmented, spore mass difficult to see within, wall difficult to break open. Encysted rods straight or slightly curved with squarish ends, 0.6 × 2.5–4.0 μ, strongly adhering together in sheaths or elongate groups, some

quite resistant to aniline-blue dye. Vegetative cells cylindrical, rounded at either end, 0.7–0.9 × 4–7 μ.

NEOTYPE: Specimens labeled *Cystobacter (Myxobacter) simplex*, collected and determined by Thaxter in October, 1892, at Waverly, Massachusetts, *Thaxter 4507*, in the Farlow Herbarium.

Material examined: One collection of Thaxter's material now in the Farlow Herbarium; the collection consists of one small piece of a polypore on which less than a dozen fruiting bodies occur. Three collections on pieces of bark gathered from living trees in Missouri and placed in moist-chamber culture.

Recorded collections: *Thaxter*, Kittery Point, Maine, and Belmont, Massachusetts, on very wet wood and bark in partly dry pools, 1893; Thaxter, Waverly, Massachusetts, on polypores, 1892 (unpublished). Peterson, on bark of living *Ulmus americana* growing at the Pinnacles, Missouri, on bark of a living *Quercus alba* growing at Holstein, Missouri, and on bark of *Quercus ellipsoidalis* growing at Duly Mill, Missouri; such bark gathered and placed in moist-chamber cultures in the laboratory.

One collection of *Haploangium simplex* is on deposit in the Farlow Herbarium of Harvard University. It is not labeled "type," but it was one of Thaxter's specimens and, therefore, can be considered representative. The collection consists of about one dozen fruiting bodies on a small piece of polypore. These specimens cannot be a part of Thaxter's original collection, because he describes that as being on wet wood and bark in partially dried pools. Therefore, this is designated as neotype material.

Haploangium ochraceum (Krzemieniewska and Krzemieniewski) comb. nov.

Polyangium ochraceum Krzemieniewska and Krzemieniewski, Acta Soc. Bot. Polon. **4**: 34. *pl. 5, f. 50, 51.* 1926.

Fruiting body a single cyst bounded by a thick, yellow-brown wall, orange to bright red in color, spherical to oval in shape, 60–80 × 50–130 μ. Contents of the cyst often divided by the wall penetrating deeply into the center of the cyst; division particularly apparent in side view; often resembling a species of *Archangium* rather than of *Haploangium* because of this characteristic of the wall. Encysted rods 0.5 × 4–8 μ. Vegetative cells not described.

LECTOTYPE: The description and photographs of *P. ochraceum* cited above.

Recorded collections: Krzemieniewska and Krzemieniewski, Poland, isolated from Polish soils and grown on sterilized rabbit dung, 1926.

As stated earlier in this paper, the writer has not studied this species. Consequently, the above description is entirely that contributed by the Krzemieniewskys. Inquiries to Madame Krzemieniewska in Poland concerning the possible existence of specimens or cultures of this and other species of their findings have not been productive. There are no known specimens of *Haploangium ochraceum* in existence and the species has been reported only on the one occasion.

Haploangium minor sp. nov.

(Etymology: Latin *minor*, less or small.)

Fruiting bodies solitary cysts, sessile in groups of 4–10, but with no contact or clustering, globose, oval or bean-shaped, 60–140 μ, turgid, smooth, dull orange-brown when fresh, collapsed, wrinkled, inconspicuous, more glistening and orange when desiccated. Spore mass not readily discernible through cyst wall; wall bright yellow-orange in transmitted light, about 2 μ thick. Encysted rods 0.7 × 2.5–3.5 μ, irregular in shape, arranged in distinct spherical groups which are difficult to break up. An amorphous material also present in the cysts. Vegetative cells with squarish ends, 0.7 × 3.0–4.5 μ.

Type material: HOLOTYPE in the University of Missouri herbarium, *Peterson 41*, on bark of *Ulmus rubra* growing in Wagner's Woods, Holstein, Missouri, Moist-chamber Culture No. 283-9. Paratypes, also on bark of *Ulmus rubra* from Wagner's Woods, Holstein, Missouri, *Peterson 42*, Moist-chamber Culture No. 283-3, in the herbarium of the State University of Iowa; and on bark of *Ulmus rubra* from Wagner's Woods, Holstein, Missouri, *Peterson 43*, Moist-chamber Culture No. 283-7, in the Farlow Herbarium.

Recorded collections: *H. minor* has been found only on bark of living trees collected in Wagner's Woods, near Holstein, Missouri, in June, 1956, and placed in moist-chamber culture. It has been found on 32 different pieces of bark from seven different trees, representing four different species. This myxobacterium has been found on eight of ten pieces of bark gathered from one *Ulmus rubra*, on seven of ten pieces from a second *Ulmus rubra*, and on seven of ten pieces of bark collected from a third *Ulmus rubra* tree. *H. minor* has also been found on one piece of *Quercus alba* bark, on six pieces of *Quercus rubra* bark, all from the same tree, and on three pieces of bark collected from two different *Fraxinus americana* trees. The frequency of fruiting bodies ranged

from only two or three fruiting bodies on some pieces of bark to uncountable numbers on other pieces.

The fruiting bodies of *H. minor* are solitary cysts in practically all cases, although an occasional clustering of two or more to give a *Polyangium*-like effect can be observed. The solitary cysts are usually found in groups of from four to ten close together, as if they all arose from the same vegetative cell mass, but there is no contact between the individuals and no connection between them. The cysts are sessile on the substrate with no suggestion of a hypothallus or stalk and they are not tightly appressed or attached to the substrate.

When mounted, stained with aniline blue, and studied under high magnifications, the cysts are seen to contain spherical masses of encysted rods and an amorphous material. The spherical masses of encysted rods are the most distinctive characteristic of *H. minor*. Sometimes these masses are perfect spheres but more often they are somewhat irregular. In either event, the rods are tightly packed into the spherical masses and are difficult to separate. Nothing is known of the amorphous material. It does not appear to be oily. It does not stain as heavily as do the encysted rods, but it will take a small amount of aniline blue and become light blue in color.

Cysts of *H. minor* were seeded onto 12 different substrates in an effort to culture the organism. Grayish-yellow colonies were produced on homogenized-bark agar and on rabbit-dung-pellet preparations after four days. Examination showed these colonies to be composed of myxobacterial cells and at least one representative of the true bacteria. In all cases, the true bacterium dominated and eventually no myxobacterial cells could be found. Attempts to transfer and purify the myxobacterial cells were always unsuccessful and further seedings with new cysts on new substrates only repeated the mixed-culture situation. The myxobacterial cells, which the writer feels confident were the vegetative cells of *H. minor*, could be measured and observed, but they could not actually be cultured, nor would they complete their life cycle and produce fruiting bodies.

Haploangium rugiseptum sp. nov.

Etymology: Latin *ruga*, wrinkled; Latin *saeptum*, enclosure.)

Fruiting bodies solitary, globose-to-oval cysts, sessile on substrate, glistening orange-red, heavily wrinkled when dry, up to 200 μ in size, but averaging about 85 μ. Cyst wall of two indistinct layers, the inner smooth and yellow, the outer irregular, flaky and dark orange. Fatty globules and amorphous material sometimes within the cyst in addition

to the encysted rods. Encysted rods 0.8 × 4–6 μ, curved once or twice, appearing knobby along their length. Vegetative cells not described.

Type material: HOLOTYPE, *Peterson 51*, in the herbarium of the University of Missouri; on bark of *Juglans nigra* collected from a tree growing in the Ashland Wildlife Area, Ashland, Missouri, Moist-chamber Culture No. 202-d-2-1. *Paratypes* on bark of *Populus deltoides* collected at Duly Mill, Missouri, *Peterson 52*, Moist-chamber Culture No. 215-b-2, in the herbarium of the State University of Iowa; and *Peterson 53*, on bark of *Quercus alba* collected in the Ashland Wildlife Area, Ashland, Missouri, Moist-chamber Culture No. 204-a-2, in the Farlow Herbarium.

Recorded collections: Known only on bark collected from living trees and subjected to moist-chamber culture. *H. rugiseptum* has been found on 27 pieces of bark collected from 16 different trees representing 10 different species in nine different geographical locations in Missouri. Although not absolutely restricted to bark from trees in such habitats, *H. rugiseptum* was preponderantly found in shady, moist situations. Moreover, much greater profusion of fruiting bodies per piece of bark occurred on pieces of bark from trees which grew in such situations than on trees in drier, less favorable habitats.

The fruiting bodies of *H. rugiseptum* are individual, sessile cysts which occur without any apparent hypothallus-like attachment. They are extremely easy to remove from the substrate and, in fact, are prone to flip away from the dissecting needle at the slightest touch. In reflected light, the fruiting bodies are bright, glistening orange-red. When immature they are quite plump, relatively unwrinkled, and more orange than red in color, but when fully mature and desiccated they are very wrinkled and more red than orange. The wrinkles on mature cysts are so prominent that they are readily seen at low magnifications under the stereoscopic microscope and it is this characteristic which suggested the specific epithet given to the organism.

When mounted and studied under the compound microscope, the heavily-wrinkled wall is striking, but it cannot be considered the outstanding characteristic for this species because the cysts of other myxobacterial species will collapse and present a similar appearance when mounted under certain conditions. The two-layered character of the cyst wall is distinctive for *H. rugiseptum*, although it is not always strikingly definite. The inner layer is bright yellow, smooth and fairly tough. The outer layer is flaky, dark red-orange and easily broken into fragments. The encysted rods within the cyst are completely free of each other and are not arranged in any particular pattern. The lack of

arrangement of the rods within the cyst is an important point because two of the other species of the genus, *H. simplex* and *H. minor*, are characterized by rods in distinctive patterns. Cysts containing striking amounts of oily globules have been observed, but this is not a constant character and other cysts with no trace of oil globules have been found on an equal, or greater, number of occasions.

Cysts of *H. rugiseptum* were sown on seven different substrates and placed in five different hanging-drop preparations in an effort to culture the organism. Such attempts were repeated and repeated, but at this writing *H. rugiseptum* has not been cultured and the vegetative cells have not been observed.

The outstanding diagnostic characteristics of the four species comprising the genus *Haploangium* can best be summarized and compared by the following key:

1. Encysted rods in definite, distinctive packets
   2. Encysted rods in sheaths, or elongate masses; cysts orange-red to yellow ..... *H. simplex*
   2. Encysted rods in spherical, or globose, masses; cysts brown to orange-brown in color ..... *H. minor*
1. Encysted rods random and loose in cyst
   3. Cyst wall strongly invaginated to divide the cyst contents ..... *H. ochraceum*
   3. Cyst wall heavily wrinkled; indefinitely composed of a smooth, inner layer and a flaky, outer layer ..... *H. rugiseptum*

The writer has seen three different solitary-cysted myxobacteria. He has studied a sufficient number of specimens to be convinced that there are at least three different species of myxobacteria which produce single-cysted fruiting bodies. Since the deeply-penetrating cyst wall described by the Krzemieniewskys is considered a distinctive character, a total of four species of solitary-cysted myxobacteria are known.

It is entirely possible that the above-described characteristics may not prove to be constant in nature, or on continuous culture, and that two or more of the four species treated in this paper may be variations of one species. Absolutely nothing is known of the developmental morphology of this group of organisms. The vegetative cells of only two of these four species have been observed; such observations were so cursory as to be almost useless. Nothing is known of the physiology of any of these species. Even if these shortcomings are kept in mind, there are definitely myxobacteria that produce fruiting bodies consisting of a solitary cyst bounded by a definite, persistent wall and containing encysted rods. Those myxobacteria that do produce such fruiting bodies can be most logically described as belonging to the genus *Haploangium*. At this writing, there appear to be sufficient constant, different charac-

teristics of the fruiting bodies of these myxobacteria to designate four distinct species within the genus.

SUMMARY

A new genus, *Haploangium*, of the family Polyangiaceae (Myxobacterales, Schizomycetes) has been erected to encompass those myxobacteria which produce fruiting bodies consisting of a single cyst with definite, persistent walls. Two species, *Haploangium simplex* and *Haploangium ochraceum*, both of which have long been known as members of the genus *Polyangium*, have been transferred to this new genus. Two undescribed species, *Haploangium minor* and *Haploangium rugiseptum*, both recently found on the bark of living trees placed in moist chamber cultures, are described and placed in this new genus. The meager knowledge concerning all four of these species of the genus *Haploangium* is compiled.

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HOST-SELECTED VARIATION IN STREPTOMYCES PHAGE[1]

S. Gaylen Bradley

(with 3 figures)

INTRODUCTION

The relation between host and parasite is frequently so intimate as to impress upon the reactants behavioral alterations persisting beyond their association. Enhancement of virulence for one host compared to another by "adaptation" is an example of host-parasite interaction familiar to virologists. For bacteriophage, several mechanisms are known or can be postulated to account for differences in virulence between virus infecting a cell and virus produced by a cell. Virulence here is defined as relative plaque-forming efficiency. Modification of virulence of resulting viral populations could involve: 1) non-hereditary host-induced modification of a bacteriophage by its previous host (Luria and Human, **1952**; Bertani and Weigle, **1953**); 2) hereditary host-induced modification of infecting virus; 3) hereditary modification by host-selection for particular mutants; or 4) changes in the constitution of the viral population resulting from induction by a superinfecting virulent virus of a temperate virus carried by a lysogenic host (Rountree, **1956**). This diversity of modifying mechanisms is possible for the microbial cell-phage system because gross production of virus by a single host cell represents 6–8 intracellular generations, each susceptible to alterative or selective influence by the host.

This paper is concerned with the observation that the relative virulence of streptomyces phage WSP-3 for a particular host depends upon the prior host, and with analysis of the origin of this host-influenced variation in virulence.

MATERIALS AND METHODS

Phage. Streptomyces-phage WSP-3 (P3) was isolated by adding a Wisconsin soil sample directly to a broth culture of *Streptomyces*

[1] This work has been supported by a grant-in-aid of research from the Medical Research Fund, Graduate School, University of Minnesota.

griseus strain WAc-104 (S104) shaken overnight at 30° C, and was purified by serial replating of plaques. Mass lysates were obtained by inoculating 10^3 to 10^5 phage particles into peptone-yeast-extract-broth cultures of germinated spores incubated at 25–30° overnight on a reciprocal shaker. Lysates representing single viral growth-cycles were harvested from mixtures of equal numbers of phage and germinated spores in peptone-yeast-extract-broth shaken for 3–4 hours at 30°. The latent period for P3 is 135 minutes and the average burst size is 218. Phage suspensions were clarified by centrifugation and rendered bacteriologically sterile by shaking with chloroform.

TABLE 1

HOST-RANGE OF SUBSTRAINS OF P3 AND TEMPERATE STREPTOMYCES PHAGES

Host	Streptomyces-phage*						
	Substrains of P3			Temperate phages from			WSP-2
	S11	S86	S104	S11	S86	S104	
Streptomyces griseus							
S86	+	+	+	+	+	+	−
S104	+	+	+	+	+	+	+
WAc–34	−	−	−	−	+	−	+
1945	−	−	−	+	−	−	+
3511	−	−	−	−	−	−	+
S. olivaceus							
S11	+	+	+	+	+	+	+
S. cyaneus							
WAc–45	−	−	−	−	−	−	+

* Lysis is denoted by + and non-lysis by −.

Plaque-counts. Stock cultures of sporulating streptomycetes were grown on AMC agar (Bradley and Lederberg, **1956**). For discrete viral plaques, appropriate phage dilutions were plated with fragmented aerial hyphae on 1.5 percent Bacto-agar medium containing 0.3 percent Bacto peptone and 0.2 percent Bacto yeast-extract.

RESULTS

Influence of preceding host on phage virulence. The purified parental strain of phage P3 grown on S104 produced plaques on other streptomycete strains. Different titers, *i.e.*, different degrees of virulence, were exhibited with some heterologous indicator hosts. For investigation of this phenomenon, relative virulence was expressed in

whole numbers as relative plaque-count, *i.e.*, by the ratio of plaque-count on the most sensitive host to the plaque count on the least sensitive host. P3 previously grown on S104 showed a higher titer with the homologous streptomycete strain than with *S. griseus* strain WAc-86 (S86) or *S. olivaceus* strain WAc-11 (S11). P3 cultivated on S11 or S86 for several cycles gave higher titers with the respective homologous host than with the heterologous hosts.

Three substrains of P3 were developed by serially replating plaques on S11, S86 or S104, respectively: these substrains are designated by notations such as "P3(104)," indicating in this case a P3 population last grown on S104. The host range of these substrains is indicated in TAB. 1. Each substrain was considered stable when the typical virulence-pattern, expressed as relative plaque-counts on S11, S86 and S104 (TAB. 2) was constant through at least 4 replatings from a single plaque.

TABLE 2

RELATIVE VIRULENCE OF 3 SUBSTRAINS OF STREPTOMYCES PHAGE P3

Prior host	No. replatings from a plaque	Relative plaque-count on		
		S11	S86	S104
S11	9	1000	1	10
S86	9	1	10,000	100
S104	9	100	1	10,000

The influence of the prior host of P3 on virulence-pattern is shown in FIG. 1. A single growth-cycle of P3(11) in S104 was sufficient to convert the typical P3(11) virulence-pattern into one corresponding to that of P3(104). The virulence-pattern of P3(104) did not revert to that of P3(11) after a single growth-cycle in S11; rather, a higher titer on S104 than on S11 resulted. Two to four cycles of growth on S11 were required to reduce the titer on S104 to equal that on S11 and only after 6–8 burst-cycles in S11 was the typical P3(11) pattern re-established. The abrupt alteration of P3(11) virulence-pattern by a single growth-cycle on S104 persisted for 6 burst cycles. This represents 36 phage-generations, on the basis of approximately 6 generations per burst. The virulence-pattern of P3(86) also was altered sharply by a single growth-cycle in S104 and was progressively returned to the typical P3(86) pattern by a few growth-cycles in S86. The modified virulence-patterns was hereditary and therefore not the result of non-hereditary host-induced modification.

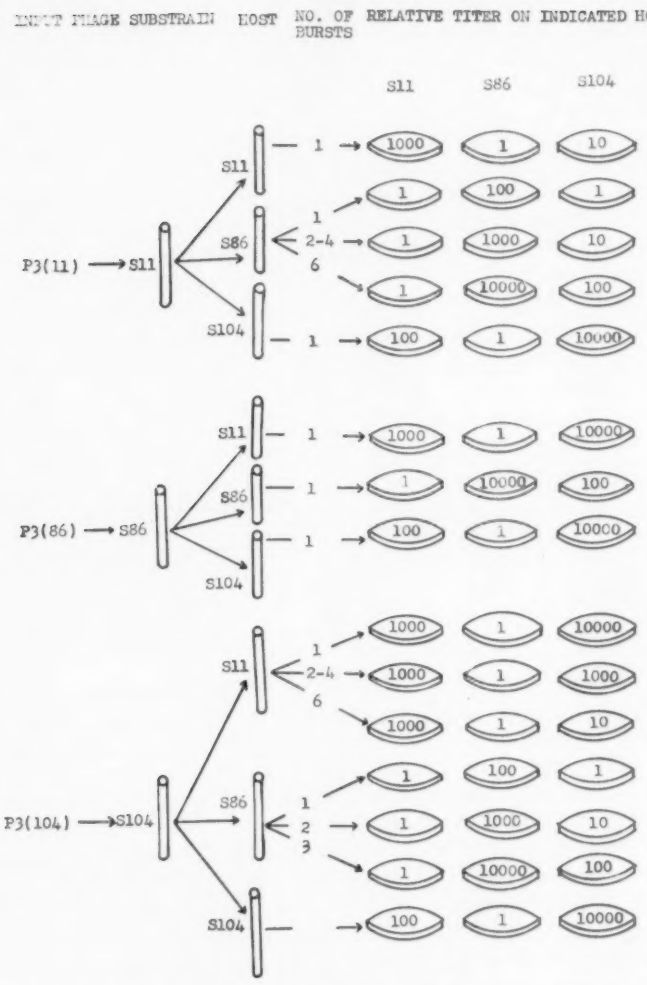

FIG. 1. Modification of virulence-pattern of Streptomyces phage P3 for *Streptomyces olivaceus* S11, *S. griseus* S86 and *S. griseus* S104 after growth on different hosts.

Possible role of temperate phage in modifying virulence-pattern. S11, S86 and S104 were each lysogenic. Free phage occurred in low numbers in autolyzing broth cultures and could be enriched by adding susceptible germinated spores. These temperate phages were regularly obtained from lysates of streptomyces phage WSP-2, which acted as an inducer (Bradley, **1957**). The temperate phages were purified by replating on WSP-2 resistant hosts. According to host range (TAB. 1) the induced phages derived from S11 and S86 were distinct from P3 while the induced phage from S104 was not differentiated.

TABLE 3

SUSCEPTIBILITY OF SUBSTRAINS OF P3 AND TEMPERATE STREPTOMYCES PHAGES TO ADVERSE CONDITIONS

Treatment	Streptomyces phage					
	Substrains of P3			Temperate phages from		
	S11	S86	S104	S11	S86	S104
Osmotic shock						
Percent survivors	23	23	26	62	43	21
Ultraviolet irradiation						
Percent survival after 10 seconds	29	27	26	5	13	29
Heat treatment						
Percent survival after 10 minutes	96	99	97	12	67	84

If the substrains with their different virulence-pattern were in reality mixed populations of dissimilar temperate phages induced by superinfection of S11, S86 or S104 with P3, the identical host ranges exhibited by the purified substrains (TAB. 1) would not be expected. Further evidence of the close similarity of P3 substrains was provided by selection of mutants of S11, S86 and S104, respectively, resistant to infection by P3(11) or P3(104); selection for resistance to one P3 substrain conferred resistance to the other two substrains. To rule out population equilibria involving other undetected temperate phages, the three P3 substrains were examined for differences with respect to inactivation by heat, ultraviolet irradiation and osmotic shock. Heat resistance was determined at 60° C and pH 7.0 (Welsch and Minon, **1955a**); survival to osmotic shock was measured after rapidly diluting a phage suspension which had been slowly adjusted to 40% sucrose (Anderson, **1950**); and ultraviolet sensitivity was ascertained by progressive exposure of phage to two 15-watt germicidal tubes, 40 cm above

the irradiated surface (Welsch and Minon, **1955b**). The close similarity of the P3 substrains was confirmed by these experiments (TAB. 3) and by tests for immunological relation, all of which readily distinguished other streptomyces-phages (FIG. 2). Dynamic population equilibria of pre-existing phages then did not account for the variation in P3 virulence-pattern.

Influence of host-selection on virulence modification. Direct evidence of selection was obtained from frank mixture experiments. Phage

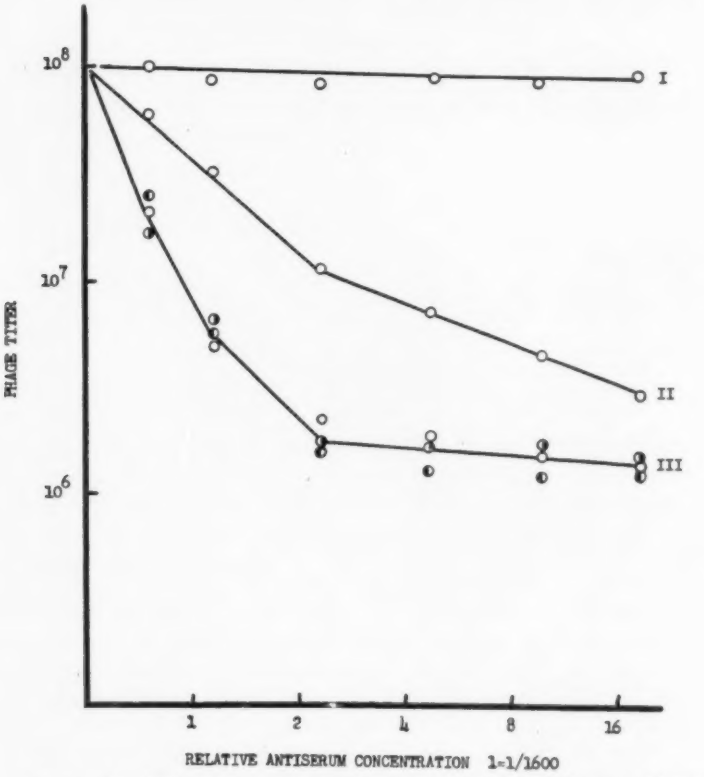

FIG. 2. Inactivation of Streptomyces phages with P3(104) antiserum. Curve I shows that the temperate phage from S11 was not affected by anti-P3(104). Curve II shows some cross reaction between anti-P3(104) and the temperate phage from S104. Curve III shows that P3(11), ◑; P3(86), ◐; and P3(104), ○; react equally.

TABLE 4

RELATIVE VIRULENCE OF FRANK MIXTURES OF P3(11) AND P3(104) FOR S11

No. phages per spore*		Titer on S11 / Titer on S104	
P3(11)	P3(104)	At 0 time	After 1 growth cycle
1	0.5	0.03	2
0.5	1	0.005	0.4
1	1	0.01	0.9
1	2	0.005	0.4
1	5	0.002	0.1
1	10	0.001	0.05
1	20	0.0005	0.02
2	1	0.02	1.6
5	1	0.05	3
10	1	0.1	5
20	1	0.2	7

* Phage titers determined on S11.

populations equal in number but composed of different proportions of the P3(11) and P3(104) sublines were added to spore suspensions of S11. Except in two experiments, the multiplicity of infection was at least one of each type of phage particle per spore. Although the incidence of mixed infection could not be tested directly, multiple absorption was revealed by decrease in concentration of each free phage. Mixed infection of S11 has been demonstrated with phage WSP-2 and P3 (Bradley, unpublished results). The relative virulence for S11 and S104 of the phage populations derived from the frank mixtures (TAB. 4) differed markedly from the ratios of titers expected on the basis of relative concentration of virus types originally added to each mixture. In all instances, the final virulence-pattern of the resulting phage was more like that of P3(11) than was the pattern of input phage.

DISCUSSION

This report presents evidence for host-selection of streptomyces-phage mutants of differing virulence. What seemed to be typical non-hereditary host-induced modification of virulence was obtained when phage P3 grown on *Streptomyces griseus* (S104) was then grown for 2–3 cycles on *S. olivaceus* (S11) (FIG. 3). The phage so derived exhibited equal titers with S11 and S104. Phage grown 3–4 cycles on S11, after a passage on S104, also gave equal titers with S11 and S104. Conversely, phage from a single burst on S104 gave a titer 100-fold higher on S104 than on S11. However, phage grown for 6 to 8 burst

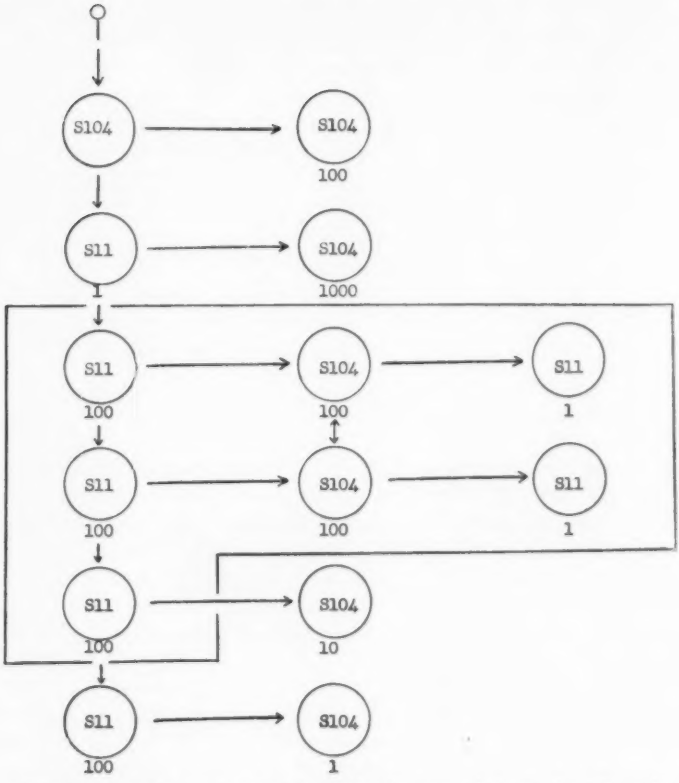

FIG. 3. Hereditary host-selected modification can mimic non-hereditary host-induced modification. The numerals below each figure represent relative plaque-forming efficiency. The enclosed portion resembles non-hereditary host-induced modification.

cycles on S11 was more virulent for the homologous host. This modification of virulence pattern was hereditary.

Since the employed streptomycete strains were lysogenic, observed shifts in relative virulence might have resulted from dynamic equilibria between temperate phage populations. P3 substrains of differing virulence-pattern selected by serially replating plaques on the same host did not show host-range differences exhibited by two of the temperate phages derived from the lysogenic host strains. When the P3 substrains could not be differentiated by additional criteria, the hypothesis

of temperate phage interaction was discounted as a mechanism of variation in virulence-pattern.

Mutation and selection seemed to be the most probable mechanism. Selection was directly demonstrated by frank mixture experiments. Operation of mutation was expected to produce considerable sample variation in the plating efficiency of single bursts of single spores; such variation was not observed. This lack of variation might have resulted from strong intracellular selection.

If certain genetic and physiological conditions of host and parasite are not compatible, intimate interaction cannot be established; once established such intimacy may facilitate heritable alteration of both host and parasite.

SUMMARY

The relative virulence of the streptomyces phage WSP-3 for *Streptomyces griseus* strains WAc-86 and WAc-104 and *S. olivaceus* strain WAc-11 depended upon the prior host. Modification of virulence pattern was hereditary, discounting non-hereditary host-induced modification. Although the hosts were lysogenic, population equilibria among different temperate phages were not involved in the shifts of relative virulence produced by the preceding host. Hereditary modification of the virulence-pattern resulted from selection of mutants of different virulence.

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GENETICS OF COCHLIOBOLUS HETEROSTROPHUS. I. VARIABILITY IN DEGREE OF COMPATIBILITY [1]

R. R. NELSON [2]

It was reported previously that *Cochliobolus heterostrophus* Drechsl. (*Helminthosporium maydis* Nisik. & Miyake) is heterothallic, in that monoconidial and monoascosporic isolates are self-sterile and cross-fertile (1). Furthermore, the species comprises 2 distinct compatibility groups, designated as *A* and *a*, all isolates exhibiting complete intergroup fertility. Certain monoconidial and monoascosporic isolates of the same compatibility group occasionally are fertile when mated with one another, although the occurrence of intragroup fertility is rare and no two isolates of the same group consistently produce fertile perithecia when paired with each other. Subsequent research indicated that compatibility in *C. heterostrophus* is controlled by a single major gene locus, although it was recognized that modifying or secondary genes undoubtedly influence the gene locus for compatibility, while other gene loci control additional steps in the sexual process of the fungus (2).

With a continued study of intergroup and intragroup fertility, it became apparent that conidial isolates within a given compatibility group differ from one another in their degree of compatibility. Such differences could be measured in several ways. The results of those studies are reported herein.

METHODS AND MATERIALS

The methods and materials used in these studies were similar to those described previously (1). Cultures derived from single conidia or single ascospores were mated by placing small pieces of mycelium on opposite sides of a section of sterile corn leaf placed in the center of a Petri plate containing Sach's nutrient agar. All matings were incubated at 24° C.

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Monoconidial isolates of the same compatibility group were compared by determining: 1) the relative frequency of non-fertile matings between known compatible isolates; 2) the degree of compatibility of isolates with relation to their frequency of fertile intragroup matings; and 3) the number of mature and immature perithecia produced in different intergroup crosses.

RESULTS

Frequency of fertile intergroup matings: The frequency with which isolates mated with known compatible isolates was determined for 10 isolates of each compatibility group. The isolates studied were those

TABLE I

RELATIVE FREQUENCY OF FERTILE CROSSES WITH COMPATIBLE ISOLATES OF COCHLIOBOLUS HETEROSTROPHUS

Conidial isolate	Percentage of fertile intergroup crosses[a]	
	Group *A*	Group *a*
1[b]	96	98
2	94	91
3	88	91
4	87	88
5	81	85
6	80	72
7	69	71
8	65	70
9	64	65
10	58	62

[a] Each isolate was studied in 150 or more intergroup pairings.
[b] Isolates of both compatibility groups, *a* and *A*, are numbered 1–10.

that had been used most often in intergroup pairings, each isolate having been mated at least 150 times with compatible strains. Each isolate was mated approximately 15 times with each of the 10 isolates of the opposite compatibility group.

Within group *A*, the frequency of fertile intergroup matings ranged from 58 to 96 percent and within group *a* the range was from 62 to 98 percent, indicating wide differences in degree of compatibility (TABLE I). As a result of these studies, isolates were arbitrarily designated as: (1) "Strong," if they mated with compatible isolates 90 percent or more of the time; (2) "Intermediate," if they mated 80 to 89 percent; and (3) "Weak," if they mated less than 80 percent.

Intragroup fertility: The frequency of intragroup fertile matings was determined for the 20 isolates (10 *A* and 10 *a*) listed in TABLE I. Each

TABLE II

FREQUENCY AND PATTERN OF INTRAGROUP FERTILITY AMONG 10 ISOLATES IN COMPATIBILITY GROUP *A* OF COCHLIOBOLUS HETEROSTROPHUS

	Isolates[a] and mating reaction[b]									
	1	2	3	4	5	6	7	8	9	10
1	0	0	0	0	0	0	F	0	F	F
2	0	0	0	0	0	0	F	0	0	0
3	0	0	0	0	0	0	F	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	9	0	F	F	0
7	F	F	F	0	0	0	0	0	0	0
8	0	0	0	0	0	F	0	0	0	0
9	F	0	0	0	0	F	0	0	0	F
10	F	0	0	0	0	0	0	0	F	0

[a] Isolates are listed in order of their relative frequency of non-fertile intergroup crosses. Isolate 1 was involved in the lowest percentage of non-fertile crosses; isolate 10 was involved in the highest percentage of non-fertile crosses. The isolates and their percentages of non-fertile crosses are the same as listed in TABLE I.

[b] 0—Non-fertile cross; F—Fertile cross.

of the isolates was mated 20 times with each of the other isolates of the same compatibility group.

In compatibility group *A*, 8 intragroup fertile matings involving 8 isolates were observed (TABLE II). In each fertile mating at least one of the isolates involved was a "Weak" isolate as measured by its relative

TABLE III

FREQUENCY AND PATTERN OF INTRAGROUP FERTILITY AMONG 10 ISOLATES IN COMPATIBILITY GROUP *a* OF COCHLIOBOLUS HETEROSTROPHUS

	Isolates[a] and mating reaction[b]									
	1	2	3	4	5	6	7	8	9	10
1	0	0	0	0	0	0	F	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	F	0	0	0	F
4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	F
6	0	0	F	0	0	0	0	0	F	0
7	F	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	F	0	0	0	0
10	0	0	F	0	F	0	0	0	0	0

[a] Isolates are listed in order of their relative frequency of non-fertile intergroup crosses. Isolate 1 was involved in the lowest percentage of non-fertile crosses, while isolate 10 was involved in the highest percentage of non-fertile crosses. The isolates and their percentages of non-fertile crosses are the same as listed in Table I.

[b] 0—Non-fertile cross; F—Fertile cross.

ability to cross with known compatible lines. In crosses 9 × 10 both of the isolates were "Weak." Perithecia never were obtained when 2 "Strong" isolates within a group were paired. Six of the matings were repeated successfully, although in each case several additional pairings were necessary to obtain a second fertile cross.

Within group *a*, 5 intragroup fertile matings involving 7 isolates were observed (TABLE III). At least one "Weak" isolate was involved in each intragroup fertile pairing and in cross 6 × 9 both isolates were "Weak." Four of the 5 matings were repeated successfully, although several additional matings were needed to obtain a second fertile cross.

Number of mature and immature perithecia: When the "Weak" isolates of either group were mated with either "Weak" or "Strong" compatible isolates, fewer perithecia were obtained than when 2 "Strong" isolates were mated. The length of time required for perithecia to mature was greater in crosses of "Weak" isolates than with "Strong" isolates. Larger proportions of immature perithecia usually were found in crosses of 2 "Weak" isolates. These results as well as those mentioned previously show that isolates of *C. heterostrophus* vary in degree of compatibility.

An analysis of hyphal-tip progenies from "Weak" and "Intermediate" isolates indicated that at least some isolates of the fungus are heterocaryotic for the compatibility locus. Two of 100 hyphal-tip cultures obtained from a "Weak" *A* isolate and mated to known tester lines were compatible with *A* tester isolates. Likewise, 3 of 200 hyphal-tip cultures obtained from a "Weak" *a* isolate mated with *a* tester isolates when crossed to tester lines. One hundred hyphal-tip cultures from each of 2 additional "Weak" *A* isolates and 2 additional "Weak" *a* isolates failed to demonstrate similar variation. It is not likely that heterocaryosis is responsible for the extreme differences in compatibility. If "Weak" isolates contain large numbers of nuclei that carry the gene for the opposite compatibility group, such heterocaryotic isolates also should be involved in frequent intragroup fertile crosses. The results do not support this postulation. In compatibility group *A*, the weakest isolate (isolate 10, TABLE I), was involved in only 2 fertile intragroup crosses in 180 intragroup pairings. The weakest isolate in group *a* (isolate 10, TABLE I) also was involved in only 2 fertile intragroup crosses in 180 pairings.

An evaluation of ascospore progenies from crosses of conidial isolates indicates that variation in degree of compatibility is heritable (TABLE IV). Ninety-two and one-half percent of the ascospore progenies derived from "Strong" × "Strong" crosses mated with compatible lines,

while 86.2 percent of "Strong" × "Intermediate," 77.9 percent of "Strong" × "Weak," and 62.8 percent of "Weak" × "Weak" progeny mated. Sufficient data were not available to establish the extent of mating of "Weak" × "Intermediate" crosses.

TABLE IV

RELATIVE DEGREE OF COMPATIBILITY OF ASCOSPORE PROGENY OBTAINED FROM CROSSES OF "STRONG," "INTERMEDIATE" AND "WEAK" CONIDIAL ISOLATES [a]

Conidial cross	Number of ascospores tested	Percentage of fertile intergroup crosses
"Strong" × "Strong"	341	92.5
"Strong" × "Intermediate"	273	86.2
"Strong" × "Weak"	247	77.9
"Weak" × "Weak"	194	62.8

[a] Isolates designated as: (1) "Strong," if they mated with compatible isolates 90 percent or more of the time; (2) "Intermediate," if they mated 80 to 89 percent; and (3) "Weak," if they mated less than 80 percent of the time.

DISCUSSION

The results summarized herein demonstrate that monoconidial and monoascosporic isolates of *C. heterostrophus* vary in their degree of compatibility, and further indicate that the variation is gene controlled. The marked differences in degree of compatibility within each compatibility group suggest that a number of genes control the variation. The fact that some isolates of both compatibility groups are "Weak" indicates that the additional genes are not linked to a specific compatibility group. These results contribute further evidence that the gene locus for compatibility is influenced by a number of associated genes.

With the knowledge that such variation exists within the species, further research has been concerned with determining what types of genetic variants could be obtained from crosses of "Weak," "Intermediate" and "Strong" isolates. Three of 19 ascospore progenies obtained from a recent cross of a "Weak" and an "Intermediate" isolate do not mate with either parent. These 3 isolates mated with *a* isolates and thus can be assigned to the *A* compatibility group. The inability to mate with parental isolates can be transmitted to isolates of both compatibility groups by sufficient out-crossing and back-crossing. It can be assumed that both the "Weak" and the "Intermediate" isolates used contribute some genetic factors for mating inhibition. It is likely that

these genes are part of the gene complex that governs the variability in degree of compatibility. These studies tend further to confirm the belief that the sexual mechanism in *C. heterostrophus* is complex.

SUMMARY

Monoconidial and monoascosporic isolates of *C. heterostrophus* exhibit marked differences in degree of compatibility. The most critical comparison is made by determining the relative frequency with which isolates fail to cross with known compatible isolates.

Studies of ascospore progeny of conidial crosses indicate that the differences in degree of compatibility are gene controlled.

The data suggest that the genes controlling the differences in intergroup fertility influence the major gene locus for compatibility and give added support to the postulation that the sexual mechanism in the species is complex.

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GENETICS OF COCHLIOBOLUS HETEROSTROPHUS. II. GENETIC FACTORS INHIBITING ASCOSPORE FORMATION [1]

R. R. NELSON [2]

It has been demonstrated that the genetic systems controlling sexual processes in the fungi are complex (**1**, **2**, **7**, **8**, **10**). It is possible, with the criteria frequently used, to consider certain heritable reactions, such as mating type or compatibility group, as being controlled by a "major" gene or genes. Modifying or "minor" genes may influence the major gene loci directly or may control additional steps in the sexual process. The influence of modifying genes may be masked in some instances by simple dominance or by the fact that certain expressions are controlled quantitatively.

Recent investigations of *Cochliobolus heterostrophus* Drechsl. (*Helminthosporium maydis* Nisik. & Miyake) have demonstrated that monoconidial and monoascosporic isolates belong to one of two compatibility groups, designated as *A* and *a*, and that the inheritance of the compatibility reaction is controlled by a single major gene locus (**5**). Subsequent research indicated that isolates of both compatibility groups vary in their degree of compatibility and that the variation is gene controlled (**6**). Monoconidial and monoascosporic isolates can be designated as "strong," "intermediate" and "weak," based on the relative frequency with which they cross with known compatible lines. In studying the inheritance of compatibility, the significance of the failure of compatible isolates to cross, as well as the failure of ascospore progeny to mate with parental isolates, is not evident in the final analysis of segregation ratios. Complete segregation data can be obtained by re-mating ascosporic lines to parental isolates until all progenies are evaluated.

With the knowledge that additional genes influence the expression of the major gene locus for compatibility, further research has been con-

[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the North Carolina Agricultural Experiment Station; published with the approval of the Director of Research as Paper No. 914 of the Journal Series.

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cerned with the genetic variants obtainable through hybridization and recombinations. From one cross, monoascosporic isolates were obtained that produced perithecia with sterile asci when selfed. The results of the studies with the "sterile ascus" isolates are reported herein.

MATERIALS AND METHODS

Mature perithecia of *C. heterostrophus* were obtained by placing small pieces of mycelium of compatible isolates on opposite sides of a section of sterile corn leaf placed in the center of a Petri plate containing Sach's nutrient agar. At 24° C, immature perithecia appeared in 5 to 7 days and immature asci in 12 to 15 days. A few of the asci contained mature ascospores in 20 days, and most of the functional asci contained mature spores by 25 days.

In evaluating the progenies obtained from crosses of "strong," "intermediate" and "weak" conidial isolates, monoascosporic isolates were selfed, mated to both parental isolates, and mated to a "weak" isolate of both compatibility groups. Ascospore progenies obtained from crosses of ascosporic isolates and "weak" conidial isolates were studied in the same manner.

One hundred and eighteen monoascosporic isolates were obtained from a cross of a "weak" *A* conidial isolate and a "strong" *a* conidial isolate. When mated to both parents and selfed, they segregated approximately 1:1 for compatibility and 116 were self-sterile. Two monoascosporic isolates produced normal perithecia with sterile asci when selfed. One of the 2 isolates, hereafter designated as isolate 90, was compatible only with *A* isolates, and such crosses produced perithecia containing varying proportions of sterile and normal asci. The other isolate, designated as isolate 83, crossed with *a* isolates and such crosses likewise yielded perithecia containing some sterile and some fertile asci. The results summarized in this paper are concerned only with isolate 90.

RESULTS

The production of perithecia with sterile asci by isolate 90 could be maintained by mycelial transfers of the original culture or from Petri plates of subsequent generations of the isolate. The mycelial transfers, hereafter referred to as "agar block" transfers, constituted $\frac{1}{4} \times \frac{1}{4}$ inch blocks of mycelial growth and agar. After 3 vegetative generations, some of the agar-block transfers from Petri plate cultures of isolate 90 failed to produce perithecia. All subsequent agar-block transfers from non-fertile plates were non-fertile. When agar-block transfers from

non-fertile plates were mated to *A* and *a* tester lines, only 1 compatibility group, either *A* or *a*, was recovered from each non-fertile plate.

The pattern of dissociation of isolate 90 was plotted by recording the positions of agar-block transfers taken from fertile plates producing sterile asci. Each of 25 agar-block sub-isolates, taken from fertile plates, was selfed, and the number of agar blocks producing sterile asci was recorded. These data were plotted against the original positions of the agar block transfers. A total of 40 plates, including 8 plates from each of 5 transfer generations, were studied. The results showed that, in general, the dissociation of isolate 90 followed a uniform pattern. Within any given area of a fertile plate, all agar block transfers produced sterile asci or all were non-fertile. The most frequent dissociation occurred at the outer extremities of the cultures.

The ability of isolate 90 to produce perithecia with sterile asci when selfed indicated that the isolate contained the factors for both compatibility groups in addition to one or more factors for ascospore inhibition. The fact that dissociation occurred indicated that all factors were not present within the same nucleus. Further studies were made to determine whether the factors were carried in different nuclei in the same hyphae or in nuclei of different hyphae. Fifty hyphal-tip isolates were obtained from each of 2 fertile plates of isolate 90. None of the isolates produced perithecia with sterile asci when selfed. Mating studies indicated that the isolates were divided in approximately equal numbers in compatibility groups *A* and *a*. Similar results were obtained with 100 monoconidial isolates. These data demonstrated that isolate 90 was comprised of a mycelial mixture of 2 biotypes and that all of the nuclei in a cell or in cells of the same hyphal strand were similar genetically.

Perithecia with sterile asci were obtained by pairing *A* and *a* hyphal tips of isolate 90. Sixteen *A* hyphal-tip isolates were paired with each of 9 *a* hyphal-tip isolates. All of the 144 crosses produced perithecia with sterile asci. It was evident from these studies that hyphal-tip isolates of both compatibility groups carried some of the genetic factors necessary for inhibition of ascospore formation.

Crosses of *A* and *a* hyphal-tip isolates with "weak," "intermediate" and "strong" conidial isolates produced perithecia containing sterile asci and normal asci in varying proportions. Some of the perithecia examined contained no asci, although the perithecia were normal in appearance. Monoascosporic isolates obtained from these crosses were mated back to *A* and *a* hyphal-tip isolates of 90. Of the 127 crosses studied, 102 produced perithecia with sterile asci only, 15 produced perithecia

with sterile and normal asci and 10 produced sterile perithecia with no asci. Fourteen of the 15 crosses that produced some ascospores involved ascospore isolates mated with *a* hyphal-tip isolates. With few exceptions, such crosses produced greater numbers of perithecia and asci.

DISCUSSION

It is concluded that the process inhibiting ascospore formation is controlled by more than a single gene. If ascospore inhibition were controlled by a single dominant gene, crosses of hyphal-tip cultures of isolate 90 and normal wild type isolates would produce all sterile asci. Conversely, such crosses would produce all fertile asci if the process were controlled by a single recessive gene. In all instances, however, these crosses have yielded both sterile asci and and fertile asci in varying proportions. When ascospore progeny from crosses of hyphal-tip cultures and wild-type isolates were back-mated to *A* and *a* hyphal-tip cultures of 90, the ratios of crosses yielding only sterile asci and those yielding some fertile asci appeared to fit no regular pattern. This suggests that the mechanism of spore inhibition is controlled by a number of factors.

Origin of isolate 90. The explanation for the origin of isolate 90 is merely speculative. One of the more likely possibilities is that 2 nuclei were included initially in the ascospore. Hrushovetz (3), in his studies of the cytology of ascus development in the related species *Cochliobolus sativus* (Ito & Kurib. ex Kurib.) Drechsl. ex Dast., stated that initially only a single nucleus is included in each ascospore. He observed that ascospore delimitation occurred at the 8-nucleate state, although asci contained from 1 to 8 ascospores. Similar observations were made by Shoemaker in studies with the same fungus (9). Neither worker commented on the fate of the remaining nuclei in asci containing fewer than 8 ascospores. In the distribution of the 8 nuclei following division III, 2 of the nuclei may frequently be in close proximity.

Other genetic variants, including apparently self-fertile ascospore isolates, have reacted similarly to the sterile-ascus isolates, in that dissociation and subsequent reversion to heterothallism occurred after several transfer generations. One such isolate, designated as 2, reverted to complete heterothallism after 6 selfed generations. However, 5 ascospore progenies isolated during the self-fertile generations also were self-fertile for 1 or more generations. These results tend to give added support to the postulation that more than a single nucleus may be included initially in an ascospore.

The possibility of mutation cannot be eliminated. Mutation could have occurred prior to or following spore formation. If it is assumed that isolates of *C. heterostrophus* belong to 1 or 2 compatibility groups, *A* and *a*, a mutation at any stage of development would not be evident if only a single nucleus were included in each ascospore, unless the compatibility locus also was affected. However, genetic change would be expressed if 2 nuclei carrying opposite genes for compatibility were included in an ascospore.

It is not necessary to postulate gene mutation to account for ascospore inhibition. The fact that the sterile ascus isolates were obtained from a cross of 2 normal heterothallic isolates suggests that at least some of the wild-type isolates may carry part of the genetic components needed for inhibition. Gene recombination could result in the alignment of all of the factors necessary for inhibition within a single nucleus. It is not unusual to find sterile asci in known fertile crosses of wild-type isolates.

One of the principal objectives of the present studies of the genetics of *C. heterostrophus* is to obtain additional information concerning the mechanism of sexual reproduction. From the standpoint of gene action and gene interaction little is known about the mechanism that differentiates homothallism from heterothallism. According to the presently accepted concepts, species are homothallic because they have the necessary genes for self-reproduction. Conversely, species are heterothallic because they lack one or more of the necessary genes. It is conceivable that a transition from homothallism to heterothallism in a fungus species might be dependent more on the segregation of gene factors that block steps in the sexual process than on the incorporation of genes for reproduction.

In addition to factors for spore inhibition, recent genetic crosses have produced monascosporic isolates that produce sterile perithecia with no asci when selfed. Other variants obtained fail to mate with either of their parental isolates, although they pair readily with other compatible strains to produce normal crosses. There appears to be a direct relationship between the gene factors involved in inhibition of ascospore formation, sterile-perithecium formation and backcross sterility. Sterile-perithecial strains, for example, were derived as second-generation ascospores from a cross of a sterile-ascus isolate and a wild-type isolate. Some second- and third-generation ascospore progenies obtained from crosses of parent inhibited isolates and wild-type isolates produce sterile perithecia when selfed and others produce sterile asci upon selfing.

It was reported previously that isolates within the same compatibility

group occasionally produced fertile perithecia when paired (**4**). Attempts to demonstrate heterocaryosis as a possible mechanism for this and other genetic irregularities have not been successful. The possibility exists that what is considered to be the gene locus for compatibility actually is comprised of a number of associated genes which normally are transferred as a unit during nuclear divisions. The proper gene sequence for reproduction could be obtained, theoretically, through genetic recombinations. It is conceivable that the sterile-perithecial strains represent one step in gene rearrangement. Attempts to obtain additional steps in the sexual process, as well as cytological studies of genetic variants, are in progress.

SUMMARY

A monoascosporic isolate obtained from a cross of 2 heterothallic conidial isolates of *C. heterostrophus* produced perithecia with sterile asci when selfed. Subsequent research demonstrated that the isolate was comprised of a mixture of 2 kinds of homocaryotic hyphae, one component functioning as the *A* compatibility group and the other as *a*. Genetic crosses of the 2 components always produced the sterile ascus condition. Crosses of either component with heterothallic isolates produced perithecia containing sterile asci and fertile asci. When ascospores from these crosses were back-mated to both components, sterile perithecia, perithecia with all sterile asci and perithecia with sterile asci and fertile asci in varying ratios were produced.

The possibility that 2 nuclei were included initially in the ascospore might explain the origin of the "sterile-ascus" isolate. Mutation prior to or following spore delimitation, or genetic recombination, might be the means of initiating the gene arrangement inhibiting ascospore formation.

The data reported are part of a study of the mechanism of sexual reproduction. It is suggested that the differentiation between homothallism and heterothallism might be dependent more on segregation of genes that block steps in the sexual process than on the incorporation of positive genes for reproduction.

ACKNOWLEDGMENTS

The author is indebted to Miss Louise LeMay for her assistance during this investigation.

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SOME STUDIES OF EMERICELLOPSIS

L. W. Durrell

(WITH 5 FIGURES)

During a survey of soil fungi in the Rocky Mountain region a *Cephalosporium* was frequently isolated whose perfect stage belongs to the genus *Emericellopsis* of the *Eurotiales*. When first observed several years ago it was of interest, as at that time no member of that genus had been reported from North America. Van Beyma, Malan, and Stolk have described species from Europe and Africa. More recently Cain has described a species from Canada and Grosklags and Swift described one as a perfect stage of the antibiotic-producing *Cephalosporium salmosynnematum* Roberts. Also, Routien recently isolated an *Emericellopsis* from a soil sample from Indiana.

When the Colorado form was found, the question arose whether it was new or a variant of one of the named species. To check on its identity cultures of the named species were obtained and comparisons made. This led to a more detailed study of the genus as a whole.

The genus *Emericellopsis* is a small one of five described species and one variety, as follows: *E. terricola* van Beyma—Holland soil 1937; *E. terricola* var. *glabra* van Beyma—Holland soil 1939; *E. mirabilis* (Malan) Stolk—Italian soil 1952; *E. minima* Stolk—East African soil 1955; *E. humicola* Cain—Canadian soil 1956; *E. salmosynnemata* Grosklags and Swift 1957.

These forms have all been adequately described by their respective authors, and so it does not seem necessary to repeat the complete descriptions. As the writer has accumulated additional data on the representatives of the genus over several years it may be worth while to submit them as an amplification of our knowledge of *Emericellopsis*.

In addition to the preceding species and their isolates there are those Colorado isolates used in the studies, Routien's isolate from Indiana soil and *Cephalosporium salmosynnematum* from the American Type Culture Laboratory, Washington, D. C. Some of the cultures studied were obtained through the courtesy of the Centraalbureau voor Schimmel Cultures, Baarn, Holland.[1]

[1] I wish to thank the following for their kindness in furnishing cultures: Dr. C. W. Hesseltine, of the Northern Utilization Research Branch, U.S.D A.;

The following descriptions briefly present the cultural characters of *Emericellopsis* species and some of their varieties, enough at least to indicate their variability and overlapping resemblances. All cultures illustrated are on P.D.A. FIG. 1 shows some of these cultural characters.

E. terricola. This species is usually Ochraceous.[2] The colony is wet and appressed with tongues of synnemata in clockwise whorls usually fruiting sparsely with small ascocarps. When old some strains darken to Tawny and are covered with dark ascocarps. Several variants were obtained; some of these were lanose, resembling *C. salmosynnematum,* others Pale Flesh Color, identical to *E. terricola* var. *glabra.* Another was Terra-cotta that discolored the agar, and looked like the Colorado isolate. Another variant was Mikado Brown and still another was a dirty white with tufted center.

E. terricola var. *glabra.* This variety was quite constant in character with few variants. The colony closely resembles *E. terricola* except for its lighter color, Salmon Buff with smooth surface, fruiting sparsely with small ascocarps. One variant was nearly white, another was Light Orange Yellow to Apricot Buff with wrinkled cerebriform surface, a third was identical in color and appearance to *E. terricola.*

E. mirabilis. The stock culture of this species when young is chalky white, then may become Vinaceous Buff to Ochraceous Salmon, later becoming Gull Gray with white edges. Colonies tend to sector and fruit profusely with large ascocarps; some sectors are sterile, Olive Gray in color. A number of variants have developed from this species, some with ascospores much larger than the parent strain. The variants range from Shell Pink to Fawn Color, Capucine Buff, Vinaceous Buff, Ochraceous Salmon, Verona Brown to Deep Olive Gray and Deep Dull Gray. Several of these variants closely resemble the Colorado isolate and *E. humicola.* Some are apparently sterile.

E. minima. The stock colony develops a smooth wet surface growth, Ochraceous Salmon to Ochraceous Tawny; sometimes the surface is tufted. It is a very rapid grower, bearing sparse small ascocarps. A few variants developed, ranging from white to cream, with swirled

Dr. John B. Routien, Chas. Pfizer & Co.; Dr. Roy F. Cain, University of Toronto; Dr. Myron P. Backus, University of Wisconsin; and Dr. Johanna Westerdijk, Centraalbureau voor Schimmel Cultures. My gratitude is expressed to Dr. J. C. Gilman, Iowa State College, and to Dr. M. W. Gardner and Dr. W. C. Snyder of the University of California for their kindness in reading the manuscript. My thanks are due also to Dr. Ralph R. Baker and Dr. Robert V. Parke of Colorado State University for valuable assistance.

[2] All colors referred to are from Ridgway's Color Standards and Nomenclature.

FIG. 1. Cultures of different species and variants of *Emericellopsis:* 354, Colorado strain; 354/1, variant of Colorado strain; 362, smooth and rough cultures of *E. terricola;* 362/1 and 362/2, variants of *E. terricola;* 363, stock culture of *E. terricola* var. *glabra;* 363/1, variant of *E. terricola* var. *glabra;* 364, *E. mirabilis;* 364/1, 364/2, 364/3, variants of *E. mirabilis;* 365, *E. minima;* 366 and 366/1, variants of Colorado strain; 367, another variant of Colorado strain; 368, *E. humicola;* 368/1, variant of *E. humicola.*

synnemata like *E. terricola,* to Pale Yellow Orange to Tawny. One variant had a lanose surface like the Indiana isolate. Another had a profusion of ascocarps in a dense central mass quite unlike the parent stock.

E. humicola. The stock culture is white to Storm Gray. The colony growth is quite restricted and tends to sector and bears ascocarps profusely. On "Complete Media" colonies are Pinkish Cinnamon. A few variants developed, ranging from Pale Olive Buff to Tawny Olive and Mikado Brown. They were all sterile.

Indiana Isolate. Stock culture Pale Salmon Color. Surface velvety, wrinkled, with erumpent centers. One variant was white and crinkled, resembling *E. humicola,* another white to Neutral Gray, darkening the agar. On "Complete Media" this became Pale Ochraceous Salmon.

E. salmosynnemata. Stock culture Pale Salmon Color to Ochraceous Salmon. Surface granular to fluffy, identical to *C. salmosynnematum,* which is a strain of the same fungus from a different source. With age all cultures look alike, a Pinkish Cinnamon, resembling the Colorado isolate. Variants out of this are cream color, resembling *E. minima,* or a velvety Shell Pink, identical in appearance to the Indiana isolate.

Colorado Isolate. Stock culture Ochraceous Salmon; colony smooth or tufted. Tends to sector profusely and produces large ascocarps. Several variants developed, ranging in color from Cinnamon Rufous to Apricot Buff and Shell Pink. Some variants had a smooth surface and colored like *E. terricola* var. *glabra;* one was lanose and granular resembling *E. salmosynnemata,* while another resembled *E. terricola.*

From thousands of cultures and numerous mutants the general impression is that the colony characters of members of the genus *Emericellopsis* are quite variable and overlap. As a means of identification they would be of little value.

Comparison was made of the different species and isolates as to growth, temperature relations, pigmentation, etc., as follows:

RATE OF GROWTH—There were differences in the rate of colony growth of the different species and isolates as indicated by colony diameter. *E. minima* grew most rapidly, quickly covering a culture plate. *E. terricola, E. salmosynnemata,* the Colorado isolate and the Indiana isolate grew more slowly. *E. mirabilis* and *E. humicola* grew slowly and restricted.

TEMPERATURE RELATIONS—The different species had an optimum growth at 25 to 30° C. There was some variation in the cardinal temperatures but nothing distinctive.

LIGHT—In contrast to the effect on species of *Fusarium,* light or

lack of it seemed to make little difference in color or growth habit of *Emericellopsis*. Duplicate sets of cultures maintained under like conditions, except light, showed little if any difference in appearance. Growth under blue light produced no differences.

PH RELATIONS—Series of cultures grown at different pH values showed no marked differences. All forms seemed to have a wide tolerance, growing well from pH 6 to 8.

PIGMENTATION—The color of the colonies of the different forms varied with the age of the culture, the medium used, and the spore strain considered. When very young the colonies were light or colorless, depending upon the medium used. Generally speaking, *E. terricola*, *E. terricola* var. *glabra*, *E. salmosynnemata*, the Indiana isolate, *C. salmosynnematum* and the Colorado isolate produced a strong orange pigment, especially with high sugar content of media. Under some conditions *E. mirabilis* and *E. humicola* also produced the same pigment, though on ordinary P.D.A. the colonies of these species are chalky to gray until ascocarps are formed, when they become nearly black. The yellow-orange pigment is a carotenoid. The range of color of the pigmented cultures, based on Ridgway's Color Charts, ranges from Pale Cinnamon Pink through Capucine Buff, Capucine Orange to Ochraceous Salmon, and from Salmon Buff through Vinaceous Cinnamon to Fawn Color and Deep Brown Vinaceous.

GROWTH ON DIFFERENT FOOD MATERIALS—The different strains of *Emericellopsis* were grown on various common media, as P.D.A., cornmeal agar, oat-meal agar, pea and bean agars, soil agar. On so-called natural media, consisting of dry tissue of various plants, little growth was evident in any culture. Ascocarps were noted on a few fragments of oat glumes.

Generally speaking, the colony character varied with the medium used. The orange pigment, as above mentioned, was strongly evident in the presence of sugars, while on less nutrient media the colonies were light-colored or colorless.

E. terricola var. *glabra* is generally lighter in color than others. Ascospore production usually occurs relatively sooner on such media as corn meal although *E. mirabilis*, *E. humicola* and the Colorado forms produce an abundance of ascocarps on a variety of media.

Czapek's medium was used with the substitution of several carbohydrates: dextrose, levulose, mannose, gallactose, lactose, maltose, sucrose, raffinose, starch and inulin. Briefly, the following results were obtained: *E. terricola* and the Colorado isolate developed the bright orange pigment on dextrose, levulose, maltose, mannose, sucrose and

raffinose. *E. minima* made the most rapid growth as noted above, produced pigment on all sugars but less intensely, and also showed faint pigmentation on inulin. *E. mirabilis* produced pigment on all sugars except lactose and gallactose; none appeared on starch. On dextrose its color was more yellow than that of other forms, being Primuline Yellow, while on other media it was Light Buff. *E. humicola* was white or colorless on all these media, though in Czapek-Dox with sucrose some yellow pigment developed. All forms made some colorless growth on starch and *E. minima, E. mirabilis* and *E. humicola* digested the starch in a wide halo about the colony. On lactose, starch and inulin, growth of all cultures was poor but on these media *E. humicola* and the Colorado isolate produced ascocarps plentifully.

It was found that *E. terricola, E. mirabilis, E. humicola* and the Colorado forms could use NO_3 and NH_3, asparagine and aspartic acid, but could barely grow on lysine. There was considerable difference in colony size and color on these different media. All four did best on NO_3 nitrogen.

Studies on amino-acid production of species and variants will be published in a separate paper. They indicate that *E. mirabilis* stands separate from the other group of species.

Among the physiological reactions of *Emericellopsis* which are of interest is its antibiotic activity. Roberts in 1952 found a substance "Synnematin" produced by *Cephalosporium salmosynnematum* that had antibiotic action. Synnematin B is a hydrophilic Penicillin resembling Penicillin W, synonymous with Cephalosporin N and Salmatin. Independently the Mycological Laboratory at the University of Wisconsin, the Lederle Laboratory and the writer noted that this imperfect stage had an *Emericellopsis* perfect stage.

The various species and variants of *Emericellopsis* react differently to bacterial test organisms. For instance, the stock culture of *E. terricola* had no effect on the test organisms *Micrococcus pyogenes* var. *aureus, Salmonella typhimurium* and *S. gallinarum,* while a variant gave a positive reaction to all three. Neither the stock culture of *E. mirabilis* nor its variant had any effect on the test organisms. The stock culture of the Colorado isolate was very active against all the above test bacteria but two of its variants had no effect on *Salmonella gallinarum* though active against the other two species of bacteria.

HOMOTHALLISM OF EMERICELLOPSIS—Preliminary observation indicated that the species of *Emericellopsis* were homothallic. Hyphal fusions have been repeatedly noted in *Emericellopsis.* Certain isolates of the fungus produced sectors that did not develop ascocarps while the

other sectors were black with ascocarps. Using all the species and isolates and several variants, tests were made that verified the above observation. The forms were grown juxtaposed in every possible combination. In no instance over weeks of time was any interrelation or even intermixing of hyphae noted, and each form maintained its homothallic character and produced ascocarps. In a few instances it appeared that some species influenced ascocarp formation from a distance but extracts of these cultures poured on the others showed no effects. The agar space between the colonies changed but a fraction of a pH, so little as to be of no influence. Pieces of this agar were dug out from between colonies and placed on other plates in front of advancing hyphae of various forms of *Emericellopsis* and were quickly overrun, apparently containing no inhibiting substance.

SINGLE-SPORE CULTURES AND VARIANTS—The experience of others with the variability of fungi suggested single-spore culturing as a means of revealing resemblances and differences of *Emericellopsis* species. This idea was strengthened by the occurrence of repeated sectoring, the sectors differing in color, growth habits and degree of sporulation. The genus appeared as a mixture with numerous segregates. At first thought it would seem that a homothallic organism such as this would remain fixed, but the work of Pontocorvo and others shows that segregations occur in such self-fertile fungi.

From some thousand single-spore cultures of *Emericellopsis* species, forms were produced repeatedly that varied in greater or less degree from the parent culture. These differences were manifest in color, texture of colony, physiological reaction, sterility or degree of sporulation and in some cases variation in spore size. The cultural differences are partly illustrated in FIG. 1 and have been discussed.

A study of the morphology of *Emericellopsis* reveals some variation between isolates in spore size and in shape and nature of the ascocarps.

Fertilization takes place in the manner characteristic of the group, starting with coiled hyphae and the development of clusters of sac-like young asci on a matrix of hyphae within an ascocarp. The asci at first are filled with granular protoplasm and soon divide into 2 then 4 and then 8 cells. The asci then separate from their cluster and enlarge as the spores mature. They usually contain 8 spores but occasionally asci with 4, 2, or even one mature spore are found. As mentioned by others, the asci are translucent and evanescent. The asci are quite constant in size; most of them are about 15 μ diameter. In *E. minima* and *E. humicola* asci may be 11–12 μ in diameter. *E. mirabilis*, however, has asci

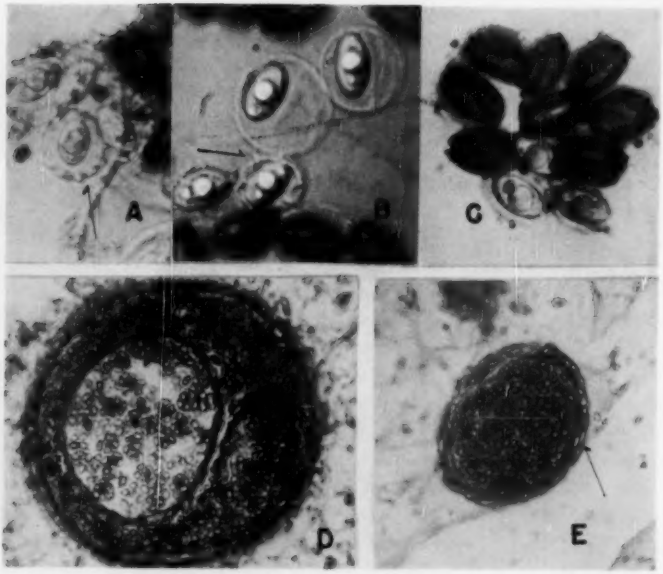

FIG. 2, a–c. *Emericellopsis mirabilis.* a and b, delicate envelope about young spores; c, envelope shrinking to become flanges of mature spore. d and e, *E. terricola.* d, peculiar development of ascocarps within an ascocarp; e, ascocarp showing thickness of wall.

some 30 μ in diameter and ascospores about twice the diameter of other forms.

ASCOCARPS—These structures are borne on the surface of the medium or are embedded in it or in some strains are borne on tufts of hyphae high above the medium. They vary in size from small ones 15 μ in diameter to many over 400 μ in diameter. In some strains the ascocarps are so small as to contain but one or two asci. The type of medium seems to effect production of ascocarps, and strains and also variants differ. One variant of *E. minima* had large ascocarps produced in dense masses. A few of the mutants obtained produced no ascocarps under any condition tried.

After fusion the ascocarp develops as a case about the asci. The walls of the ascocarp are practically the same in the different species, varying slightly with size and age. Usually they are thin, two layers of cells thick (FIG. 2e). However, they vary, being only one layer thick in small ascocarps and 3–5 layers thick in very large ones. The

cells composing the walls may be sharply angular or rounded. They overlap so that the line of juncture of two cells is covered by another cell. Grown on media containing a small amount of detergent, the hyphae are distorted and the cells swollen and the ascocarp walls are not continuous but net-like, in fact so open that the asci and ascospores as they mature and swell ooze out in black droplets through the openings in the walls. On liquid media in shake cultures ascocarps are not produced except occasionally where hyphae stick to the side of the flask.

FIG. 3. Ascospores of *Emericellopsis* species drawn to scale. These figures illustrate the flanges and oil drops characteristic of the genus. There is a difference in size of the spores but great morphological similarity. Nos. 7 and 9 are drawn from different strains of the same culture. 1, *E. terricola* var. *glabra;* 2, *E. mirabilis;* 3, *E. terricola;* 4, *E. minima;* 5, *E. humicola;* 6, *Emericellopsis,* Colorado strain; 7, *Cephalosporium salmosynnematum;* 8, *Emericellopsis,* Indiana strain; 9, *E. salmosynnemata.*

In FIG. 2d is illustrated a peculiar condition where ascocarps are produced within another ascocarp. Dozens of examples of this were found in the Colorado isolate of *Emericellopsis;* sometimes one, often two or three ascocarps were included within the original. The walls of the inner ascocarps were one cell thick and the spores took the stain differently than the spores in the outer area. This probably indicates a difference in maturity of the two sets of spores.

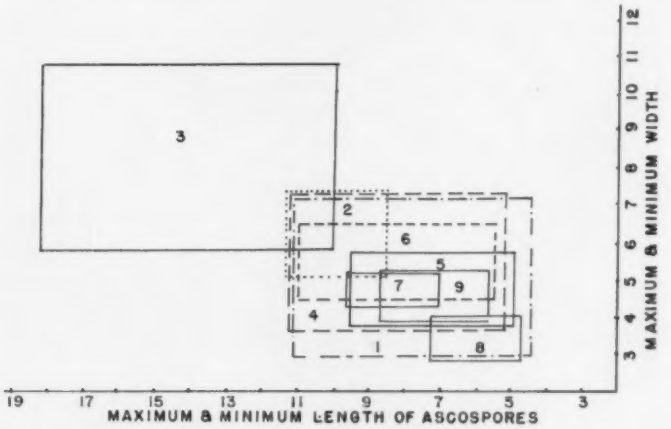

FIG. 4. Graphic representation of comparative size of ascospores of *Emericellopsis* species. Rectangle 1, *E. terricola;* 2, *E. terricola* var. *glabra;* 3, *E. mirabilis;* 4, *E. minima;* 5, *Emericellopsis,* Colorado strain; 6, *Emericellopsis,* Indiana strain; 7, *Cephalosporium salmosynemmatum;* 8, *E. humicola;* 9, *E. salmosynemmata.*

ASCOSPORES—The ascospores of all species are elliptical and usually contain a large oil drop, as evidenced by stain with Sudan IV. This drop often nearly fills the cell. The ascospores are conspicuous for the peculiar fllanges or ridges that extend lengthwise on the surface of the spore from pole to pole, often in a slight spiral, resembling other members of the *Eurotiaceae* in this respect. In FIG. 3 are shown drawings of the shape and character of the ascospores of the different species and strains of *Emericellopsis* drawn to scale for comparison.

The flanges on the ascospores vary; on smaller spores, as *E. humicola,* they are very narrow, often almost invisible even with the phase-contrast microscope. In the *E. terricola* group they range from very narrow to wide, from entire to shredded or crinkled. In *E. mirabilis,*

probably because of the larger size, the flanges are conspicuous, ragged or entire and crinkled. The flanges originate as a delicate envelope about the smooth young spore at the beginning of the 8-celled stage (FIG. 2a–c). This can easily be seen with phase contrast. At first the envelope is smooth but as the spore matures, and perhaps with some desiccation, the envelope shrinks and crinkles much as one makes creases in a felt hat. When tightly shrunken in, the edges constitute the flanges or ridges. Often a very fine stippled effect can be seen on this envelope when it is shrunken, which confers on the spore a punctate appearance

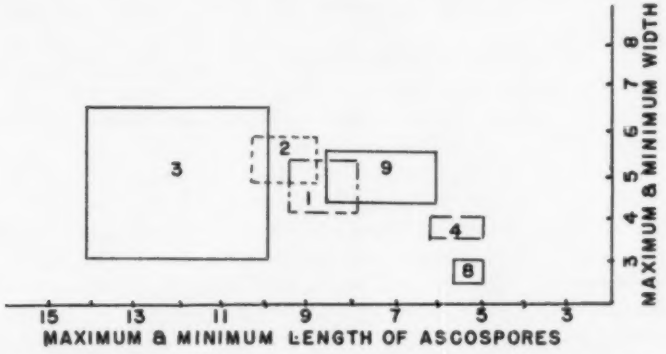

FIG. 5. Graphic representation of published dimensions of ascospores of *Emericellopsis* species. 1, *E. terricola;* 2, *E. terricola* var. *glabra;* 3, *E. mirabilis;* 4, *E. minima;* 8, *E. humicola;* 9, *E. salmosynemmata.*

(FIG. 3). This, however, cannot be seen on all the spores from any one sampling nor is it observable in all species or variants; it has not been seen on spores of *E. humicola.* Ascospores may germinate from either the end or the side.

Extensive observations and measurements of ascospores indicate that generally they are fairly constant in size within a strain or species regardless of medium. Variants, as might be expected, differ to some extent. In FIG. 4 a diagram compares sizes from different species and variants. In this chart rectangles represent the maximum and minimum lengths and widths of the ascospores.

A large number of spore measurements were made, and these indicate that the species and variants fall within two groups, the large-spored *E. mirabilis* and the small-spored group containing the other forms. The pattern differs slightly from that taken from type descrip-

tions (FIG. 5). The reason no doubt lies in the large number examined and the inclusion of figures for the mutant variants.

CONIDIA—The asexual fruiting stage of all cultures of *Emericellopsis* is of the *Cephalosporium* type. Several to many spores are characteristically clustered in heads at the tip of conidiophores. As in many species of *Cephalosporium*, the shape of the conidia varies from ovoid to elliptical to allantoid in the same culture and the same head, depending on whether the spore is inside the cluster or on the surface perimeter. On culture media containing a detergent, conidial heads seldom form or the conidiophores bear one cell like *Acremonium*. In shake cultures no heads form and the conidia are variable, often yeast-like in appearance.

The size and shape of the conidia vary about a general mean. Where the species measurements overlap to this extent it is hard to see how they can be of any value in identification, and moreover indicate the close similarity of the various forms.

In this study of the Genus *Emericellopsis* the data presented seem to indicate that all the forms studied are closely related and intergrade. They all have a *Cephalosporium* stage and are homothallic, and the general character of ascocarps and initials is the same. They all grow best between 25° and 30° and over a wide range of pH from 6 to 8. They all produce a carotenoid pigment in some degree.

The forms differ as to food preferences and the cultural characters on media are variable and the growth characters of single spore lines overlap. In amino-acid analysis *E. mirabilis* is distinct from the other group of forms. Ascocarp size varies from large to small, often intermingled in the same culture. Some variants produce no ascocarps, others produce no conidia, others are sterile. The size and shape of the conidia vary around a general average.

In *Emericellopsis* the readily recognized features are the size and character of the ascospores. In *E. mirabilis* the spores are much larger than other forms and easily recognized. The other forms vary around a general mean of characters, typical of *E. terricola*. The Indiana isolate and the colorado isolate belong to *E. terricola*.

The following synonymy is suggested:

E. TERRICOLA van Beyma

syn. *E. minima* Stolk, Brit. Mycol. Soc. Trans. **38**: 419. 1955.

E. salmosynnemata Grosklags and Swift, Mycologia **49**: 305. 1957.

Saturnomyces humicola Cain, Can. Jour. Bot. **34**: 136. 1956.

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THE INFLUENCE OF ENVIRONMENT UPON GERMINATION AND LONGEVITY OF AECIOSPORES AND UREDIOSPORES OF COLEOSPORIUM SOLIDAGINIS [1]

CHARLES L. FERGUS

An understanding of the influence of environment on the geographic distribution and intensity of rust disease is dependent in part upon determining its influence upon the germination and longevity of spores. Such information about most species of the Melampsoraceae, especially those affecting forest trees, is very meagre. However, studies of the influence of temperature and other external factors upon the spores of *Cronartium ribicola* Fischer have been extensive (**3**, **4**, **7**, **8**). In addition the influence of temperature upon germination and viability of spores of *Cronartium coleosporioides* (Diet. and Holw.) Arth. (**5**) and *Cronartium fusiforme* (Arth. and Kern) Hedgc. and Hunt (**6**) has been reported.

A plentiful collection of recently formed aecia of *Coleosporium solidaginis* (Schw.) Thuem. on needles of pitch pine, *Pinus rigida* Mill., and urediospores on leaves of *Solidago caesia* L., presented the opportunity to obtain similar information about this rust. The results are reported herein.

MATERIALS AND METHODS

Aeciospores and urediospores were removed from the sori by using a sterilized needle while observing under a stereoscopic microscope at 26 magnifications. Spores were taken only from matured open sori. The needle was then gently streaked over hardened water agar (17 g agar dissolved in one liter of distilled water) in sterile petri dishes, which in turn were placed at various temperatures in Thelco Precision incubators. Each temperature is accurate to ± 0.5 degrees. A Frigidaire freezer was utilized for the $-17°$ temperature.

Observations on spore germination at the various temperatures were made after 24 hours. In exposure tests, the dishes were removed from

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the −17°, 35° and 40° incubators after various selected intervals and incubated in the dark at 20° for 24 hours. Percent germination was then determined by observing all the spores in several low power fields (100 ×) chosen at random and recording them as germinated or non-germinated. Results are presented as percent germinated.

Some leaves and needles bearing sori were placed on porcelain trays in desiccators over $CaCl_2$ or water. The desiccators were stored at 8°. After various intervals of time the viability of the aeciospores and urediospores was determined by using the technique described above.

EXPERIMENTAL RESULTS

Only one germ tube was usually produced by a spore, rarely two, and no swelling of the spores was observed. The presence of bright orange pigment within and the large size of viable spores aided observations. The pigment flowed out into the germ tube within the cytoplasm and always aggregated in the terminal portion of the germ tube. This aided differentiation of rust-spore germ tubes from those of contaminating fungi, which were encountered only in the tests involving longevity at high relative humidities. No spore that had lost its orange color ever germinated; however, some spores that were orange failed to germinate even at the optimum temperature. Freshly formed spores are a bright orange, but observations of both aecia and uredia under the stereoscopic microscope showed that with age the percentage of colorless spores, appearing white under the stereoscope, gradually increased. The outer spores became colorless first.

The minimum time required for initiation of germ tubes was 16 hours

TABLE I

EFFECT OF TEMPERATURE UPON GERMINATION AND LENGTH OF GERM TUBES OF AECIOSPORES (I) AND UREDIOSPORES (II) OF COLEOSPORIUM SOLIDAGINIS

Temperature °C	Percent germination		Average length of germ tubes (microns)	
	I	II	I	II
3	0	0	—	—
6	38	1	37	50
10	45	56	170	200
15	65	70	200	400
20	91	70	220	220
25	84	36	202	120
30	41	1	72	250
35	0	0	—	—

TABLE II

PERCENT GERMINATION OF AECIOSPORES (I) AND UREDIOSPORES (II) OF COLEOSPORIUM SOLIDAGINIS AFTER EXPOSURE TO HIGH AND LOW TEMPERATURES

Exposure in hours	Temperature °C					
	35		40		−17	
	I	II	I	II	I	II
0	75	35	63	32	72	38
½	78	20	15	10	—	—
1½	67	10	1	0	27	22
3	10	13	0	0	20	16
4	1	10	0	0	—	—
5	0	0	0	0	—	—
8	0	0	—	—	25	14
61 days	—	—	—	—	2	1
120 days	—	—	—	—	<1	<1

at the optimum temperature. This was followed by rapid elongation, branching and occasional coiling of the germ tubes.

In two instances germ tubes of two different aeciospores were observed to fuse together.

The cardinal temperatures for germination of both kinds of spores were similar, although a greater percentage of the aeciospores germinated (TABLE I).

Both aeciospores and urediospores were very sensitive to the higher temperatures, but quite resistant to −17°. See TABLE II.

Maximum longevity of the aeciospores was 143 days stored over $CaCl_2$ and 103 days over water at 8°, and 120 and 40 days respectively for the urediospores.

DISCUSSION

The results of the influence of temperature on germination and longevity reported herein agree in general with those reported by other workers for *Cronartium* spp. (**4**, **6**, **8**). However, the constant correlation of the presence of pigment in germinable spores and its movement into the terminal growing region of the germ tube has not been previously noted. The protoplasm of the spores of many rusts contains bright-yellow to golden-yellow coloring matter which has never been fully studied. Arthur (**1**) suggests that it is held in solution by oil drops, and that it serves, like the oil, as a reserve nutrient.

In this study, only spores that contained the pigment were germinable. The bright golden color faded as the spores aged. Siggers (**6**)

and Spaulding (7) reported this change in color but only *en masse*. No other similar observations could be found in the literature. Clinton (2) germinated urediospores of *Coleosporium solidaginis* in van Tieghem cells, but made no observations about the pigment nor the effect of environment on percentage germination. The detrimental effect of sunlight on germinability of aeciospores of *C. ribicola* has been reported by both Spaulding (7) and Van Arsdel, Riker, and Patton (8); however, no clear explanation of the exact mechanism involved has been forthcoming. A study of the influence of sunlight upon the pigment might be profitable.

Exposure to high temperatures (35° for 5 hours) killed aeciospores and urediospores. Such temperatures may occur naturally in the summer and thus limit the natural spread of the rust. Less than 1 percent of the urediospores survived 120 days at a constant low temperature of −17°, indicating that the possibility of the overwintering of such spores in some localities is small, although Clinton (2) reported that urediospores of *C. solidaginis* collected in January in Connecticut germinated vigorously.

SUMMARY

The optimum temperature for the germination of aeciospores and urediospores of *C. solidaginis* is approximately 20° C. The maximum is between 30° and 35°, and the minimum is below 6°. The minimum time required for formation of germ tubes was 16 hours at the optimum temperature. The rate of growth of germ tubes was affected by temperature. A correlation between the presence of bright orange pigment in the protoplasm with germination was observed. If the pigment was not present, the spores failed to germinate, although some spores containing bright pigment also failed to germinate. The pigment was always found in the terminal extending portion of the germ tube. The germ tubes developed to considerable lengths (up to 400 μ) within 24 hours at optimum temperatures. The germ tubes of two aeciospores anastomosed.

There was no significant difference in the ability of the aeciospores and urediospores to withstand exposure to high and low temperatures. Exposure to 35° for 5 hours and to 40° for 1½ hours killed aeciospores and urediospores. Less than 1 percent survived 120 days at −17°. The longevity of aeciospores and urediospores at 8° was greater in dry than in humid atmospheres.

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CLINICAL OBSERVATIONS ON THE EFFECTS OF PANAEOLUS VENENOSUS VERSUS PSILOCYBE CAERULESCENS MUSHROOMS

SAM I. STEIN [1]

Presumably no systematic or objectively measured studies have been reported of the clinical effects of the use of the so-called hallucinogenic Mexican mushrooms (*Psilocybe*). Recently, the significant chemicals allegedly productive of these psychopharmacological effects were reported (Hofmann, **1958**).

In a clinical study with *Panaeolus campanulatus*, Child (**1952**) used 2 g of parboiled material, and reported a "noticeable intoxication" but no hallucinosis. Because of a chance relatively favorable personal experience (Singer et al., **1958**) with one variety of psychoneurophysiologically significant *Panaeolus* (probably *P. venenosus*) in contrast to an undesirable one with a 5 g amount of *P. cubensis*,[2] I had decided to proceed with my exploration of the psychoneurophysiological effects of selected mushrooms.

The following is an outline of a case study where both *Panaeolus venenosus* and *Psilocybe caerulescens* fried in butter were used in graduated amounts:

Patient J. H., age 27, under my care since April 13, 1958, because of homosexual thoughts, and in whom on first examination I had found very rapid pulse (112) and a moderate hypertension (165/90), was taking the following medication designed to control his symptoms: Sandril Con. solution, 16 drops q.i.d.; Marsalid 25 mg tablet, ½ tablet b.i.d.; Amphetamine 5 mg, ½ tablet; Amytal grain ½, 1 b.i.d. On May 4th, the subject reported a diminution of his homosexual thoughts and by May 6th he could not restore these thoughts even if he tried. On May 6th his readings were pulse 72, blood pressure 120/70, and weight 126. The Draw-A-Person test was used almost daily to substantiate his subjective claims. Positive heterosexual thoughts were asserting

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[2] Identified by Prof. Rolf Singer and grown by Prof. Leon Kneebone.

themselves only slightly. The subject was from Ireland via Canada and his visa time was becoming short. He had read of my work with mushrooms and volunteered to try the crude material in hope of expediting the effect he was seeking. In addition to the medication listed above, on May 20th, 0.5 g *P. venenosus*[2] was given without significant effect. On successive days 1 g produced positive results; 1.5 g produced a strong favorable and euphoriant effect. I have tape recordings and polygraphic tracings of some of the effects. *Psilocybe caerulescens*,[2] 1.5 g, was also tried but a different, relatively hallucinogenic, and much less pleasant effect was secured or reported. The *Panaeolus venenosus* effect was only minimally hallucinogenic and only in 1.5 g portions.

There is no doubt that the *Panaeolus venenosus* contains psychoneurophysiologic ingredient. Its effect was clearly different from that of the *Psilocybe*. Its effect was mainly stimulatory (euphoriant) and no undesirable side effects were reported or noted. The subject asked for more of the effect which was secured with 1.5 g of the *Panaeolus*. I believe this mushroom area deserves intensive experimental study and clinical exploration. I believe the chemical content or chemical arrangement in *Panaeolus* may be found significantly different from that of *Psilocybe*, since my clinical observations suggest a more favorable medicinal potential for *Panaeolus* in contrast to the mainly hallucinogenic and depressant effects obtaining with *Psilocybe*.

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NOTE ON XEROTUS AFER FR.[1]

MARIA ENEYDA PACHECO KAUFFMANN FIDALGO[2]

(WITH 2 FIGURES)

While studying the genus *Gloeophyllum* Karst. I noted that Singer (**1949**, pp. 205–206, 744) excluded the genus *Xerotus* Fr. from the *Agaricales*. According to Singer this genus is the same as *Gloeophyllum* Karst. and therefore near *Daedalea* Pers. ex Fr., *Daedaleopsis* Schroet., *Hexagona* Fr., etc. This observation is found also in Kotlaba & Pouzar's work (**1957**). Singer considers that only the type species of *Xerotus* Fr. (*X. afer* Fr.) is a polypore, the additional species included in the genus being true *Agaricales*. He transferred the latter to the genus *Anthracophyllum* Ces.

In order to clarify this question a study was made of the type material of *Xerotus afer* Fr. The type was kindly sent by Dr. Seth Lundell from the Fries herbarium to the National Fungus Collections, where I had the opportunity to examine it.

XEROTUS AFER Fr., Elenchus fungorum **1**: 48. 1828.

Type locality: Sierra Leone, Guinea, Africa. Coll. by A. Afzelius.

Macroscopic characteristics: Fruiting-body stipitate, coriaceous-membranaceous, applanate, 5 × 8 × 0.35 cm; pilear surface glabrous, slightly radially sulcate, brown-colored (R:XXIX—Verona Brown, or MP: 7A11—Vandike Brown); stipe short, hard, 1.2 cm long, 0.8 cm broad; context thin, less than 0.5 mm, lighter than the pilear surface (R:XXIX —Snuff Brown to Tawny Olive, or MP:14L9—Bronze); hymenial surface lamellate, habit lenzitoid, lamellae distant and dichotomous, 0.3 cm deep, with entire and straight edges, dark colored (R:XLV—Deep Brownish Drab, or MP:4101—Rosetta).

Microscopical characteristics: Hyphal system dimitic. The context and the dissepiments have one kind of hyphae characterized by yellow color (brownish when seen in mass), walls much thickened to solid, much branched, with limited growth, non-septate, diameter changing

[1] A study made with the aid of a grant from the National Council of Research of Brazil, at the National Fungus Collections, Plant Industry Station, Beltsville, Maryland.

[2] Naturalista of the Jardim Botanico do Rio de Janeiro, Distrito Federal, Brazil.

considerably in the same hypha, sometimes the broader part being 14 μ in diameter and the ends of the branching being only 1 μ in diam. With exception of the color, these hyphae should correspond to the type found by Cunningham (**1947**, pp. 243–244) in *Polyporus portentosus* Berk. (as *P. eucalyptorum* Fr.) which he called the "Bovista" type of skeletal hyphae. Corner (**1953**, p. 153) suggests that the best name for these hyphae is binding hyphae. The latter has shown that polypores can also be dimitic with binding and generative hyphae, and not only with skeletal and generative hyphae, as was initially figured by Cunningham (**1947**) and Teston (**1953**, pp. 81–82). In the type specimen of *Xerotus afer* remnants of generative hyphae are found with difficulty in the margin, since the latter is practically destroyed in the specimen. In this region the generative hyphae are scantily seen and seem to have been replaced by the binding hyphae. In the adjacent portion of the hymenium a few delicate filaments of generative hyphae are found, collapsed in part. These hyphae are hyaline, narrow, 1.5–3 μ in diam., not thickened, with clamps and branched. Cystidia hyaline to fuscous, thick-walled, 16.5–25 × 5–8 μ. Basidia hyaline and tetrasterigmate, 12–20 ×3.5–5.5 μ. Spores not found.

From the above analysis I agree with Singer when he states that *Xerotus afer* cannot be regarded as a genus of the *Agaricales*. Further-

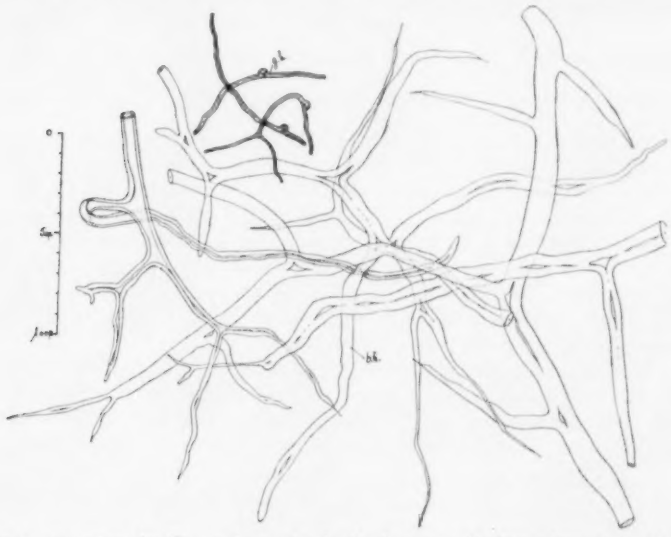

FIG. 1. *Xerotus afer* Fr. g. h., generative hyphae near the hymenium; b. h., binding hyphae of the context.

FIG. 2. Fruiting-body of the type, showing both surfaces.

more, it has none of the characteristics of *Anthracophyllum*, as this genus was emended by Singer. *X. afer* shows, for instance: no differentiated sub-hymenium; green color not developed in KOH; no carbonaceous particules present; cystidia present in the hymenium (but not in *Anthracophyllum* Ces.); context formed by a very peculiar hyphal type (binding hyphae) not observed so far in *Anthracophyllum;* hyphae with clamp connections (generative hyphae) not present in the context of the adult fruiting-body (occurring in *Anthracophyllum*), remnants of hyphae with clamps only to be found sparsely near the hymenium.

On the other hand, as I interpret the structure of *Gloeophyllum* Karst., I cannot follow Singer in his opinion that *Xerotus* Fr. and *Gloeophyllum* belong to the same natural group. Macroscopically both

genera are similar, having a lenzitoid habit and dark context, but anatomically their fruiting-bodies are so distinct that I prefer to keep them separate. *Gloeophyllum sepiarium* (Wulf. ex Fr.) Karst., the type species of *Gloeophyllum*, as indicated by Murrill (**1903**, p. 94), has a trimitic hyphal system. The examination of its context shows: generative hyphae clamped, not collapsed; brown skeletal hyphae like long fibers, walls thickened, not septate and not branched at all; hyaline binding hyphae, very narrow, moderately branched and found in restricted number (I prefer to call these as incipient binding hyphae). *Xerotus afer* seems to have a different origin from *G. sepiarium* and its relationship with other polypores is not very clear.

Among the *Agaricales, Lentinus sajor-caju* Fr. is one that shows in its context an anatomical structure similar to that of *Xerotus afer*—i.e., dimitic structure with binding hyphae. These hyphae in *Lentinus sajor-caju* are hyaline, much branched, thickened, without septa, and with a diam. from 1–6 μ. They differ from the binding hyphae of *X. afer* in a few respects, as being longer and narrower and with the ends of the branchings tapering, not narrowed in the abrupt manner found in *X. afer*.

The dimitic structure of the context and dissepiments of *Xerotus afer* seems to be a rare one, found, so far, in a very restricted number of polypores. Usually the dimitism results from the presence of skeletal and generative hyphae. *Polyporus portentosus* Berk., cited above, is one of the few species that have the same type of structure as *Xerotus*. Corner has shown dimitism with binding hyphae also in the context of *Polyporus sulphureus* Bull. ex Fr., *P. squamosus* Huds. ex Fr. and *P. betulinus* Bull. ex Fr. He holds that *P. sulphureus* has the dissepiments monomitic, *P. betulinus* has skeletal hyphae in the dissepiments, and only *P. squamosus* has the flesh and dissepiments equally dimitic with binding hyphae. The generative hyphae of *Polyporus squamosus* and *Polyporus betulinus* are septate with clamps like those of *Xerotus afer* Fr., whereas *Polyporus sulphureus* has simple cross-walls. However, no one of these species apparently has any relationship with *Xerotus afer* Fr. except, probably, *Lentinus sajor-caju* Fr.

It would be very interesting to study the structure of the fruiting-body of *Xerotus afer* Fr. in fresh material. Unfortunately this species appears to be very rare and seems to be known only from the type specimen.

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A CONTRIBUTION TOWARD LICHEN SYNTHESIS [1]

VERNON AHMADJIAN

(WITH 5 FIGURES)

The synthesis of lichens in the laboratory, beginning with the isolated algal and fungal symbionts, has long been the dream of lichenologists. Despite numerous attempts (a good history is given by Werner **1927**) and a few reported successes (Bonnier **1889**, Thomas **1939**), the problem still remains unsolved and is as enigmatic today as it was some 90 years ago when the true nature of lichens was discovered. The experiments described in this paper have resulted in some interesting observations regarding the initial stages of lichen synthesis. Several of the results obtained deviate quite markedly from those published by earlier workers, notably those of Gaston Bonnier, who has claimed to have achieved many true lichen syntheses. Bonnier described synthetic culture in which 5 days after germination the clasping hyphae had laid hold of the algae and symbiotic growth had begun, a small lichen thallus being formed some 50 days later, with subsequent apothecial formation. In the light of my experiments this is indeed a remarkable feat. Bonnier's experiments contain numerous inconsistencies and unexplained points (Thomas **1939**, p. 187); these, coupled with the fact that no one has been able to duplicate his experiments, make one hesitant in accepting his results.

The lichen used for our experiments was *Lecanora dispersa* (Pers.) Röhl., an urban, saxicolous form growing quite conveniently on the concrete ledge of the basement window of the Farlow Herbarium. The procedure, carried out under sterile conditions and using sterile apparatus and cultures, may be outlined as follows:

A non-nutrient agar (Noble's Agar) containing Knop's mineral-solution medium (pH 7.3) was employed. Immediately after autoclaving, this medium was pipetted into a van Tieghem cell and a carefully washed cover-slip was then placed over this filled cell and lifted off again

[1] Portion of a thesis submitted to the Graduate School of Arts and Sciences, Harvard University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

by means of forceps: this action leaves a thin film of the medium on the lower face of the cover-slip. As soon as this film hardened, the cover-slip was placed, with its agar surface up, on another van Tieghem cell fastened in the center of a small petri plate. After the affixing of an apothecium of the lichen to the top cover of the petri plate in a position directly above the agar surface, the plate was placed in a damp chamber. One or two days later this film contained numerous spores, usually in groups of eight, which had been forceably discharged from the apothecium.

When the spores had germinated, a suspension of algal cells taken from a clonal colony previously isolated from the same lichen was pipetted onto the agar surface. The alga, a species of *Trebouxia* inhabiting the thalline margin of the apothecium, was isolated by means of a micropipette from suspensions of crushed fruiting bodies of the lichen. Only cells having small adhering hyphal "bumps" or fragments of the mycobiont were isolated, thus avoiding the possibility of obtaining algal species growing epiphytically on the lichen thallus. The cover-slip was then transferred without fastening, agar surface down, to still another van Tieghem cell fastened to a glass slide with egg albumen and partially filled with water.

This procedure permits clear microscopic examination of the germinating spores and adjacent algal cells (FIG. 1) and subsequent drying of the film of agar enables one to view the symbionts at the higher magnifications. During part of the day (4–5 hours) the culture was kept in an incubator outfitted with fluorescent lights and a fan (temp. 26° C). The culture was shaded from the direct light by a "miracloth" cover. In the evenings the cell was transferred to a northerly exposed window of a cold room (13–15°). Despite the presence of water in the cell the culture film dried out somewhat and had to be washed daily. This was easily effected by lifting off the cover-slip with forceps and rinsing the agar surface alternately with a few drops of sterile water and Knop's mineral solution. There was no danger of dislodging the symbionts, as these become firmly attached to the agar after the first drying. In this manner the alternate wetting and drying and fluctuations of temperature that the lichen encounters in nature are simulated.

The germ tubes grew slowly and showed no chemotropic response toward the algae. Spores which germinated directly in contact with the algal cells gave no indication of a directed growth (FIG. 2).

It was only after 3–4 weeks that suggestive "unions" became evident. Some of the hyphal branches were seen to become wrapped closely

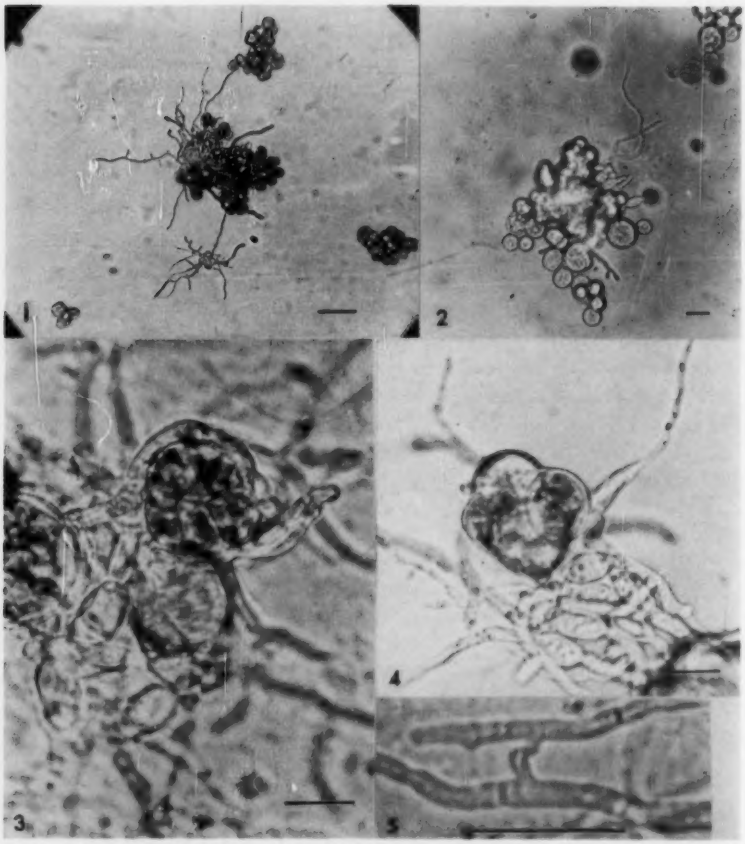

FIG. 1. Mixture of the two symbionts of *Lecanora dispersa* after 22 days (SN. 2). (Unit = 50 μ)

FIG. 2. Germinating ascospores after 10 days (SN. 2). (Unit = 10 μ)

FIG. 3. Hyphal branch encircling algal cell; note polygonal division of the alga. After 3 weeks in culture (SN. 1). (Unit = 10 μ)

FIG. 4. Fungal cell laterally appressed against an algal autospore; after 22 days growth. Four days later the cells above and below the appressed one also made contact with the same algal cell (SN. 2). (Unit = 10 μ)

FIG. 5. Hyphal fusion of lichen fungus; noted after 26 days (SN. 1). (Unit = 10μ)

around the algal cells (FIG. 3); others formed small flattened peg-like outgrowths against the cells; still others formed structures closely resembling appressoria (FIG. 4). Similar structures have been accurately reported and drawn by earlier workers (Treub **1873**, Bornet **1873**, Quispel **1943**). The important question in our investigations, however, remained unanswered; viz. were these structures functional? or was there a transfer of nutrient material taking place from the independent alga to the now rapidly starving lichen fungus? This question could be resolved by the continuing growth of these "contact" hyphae and the death of the control group of fungal mycelia not in contact with any of the algal cells.

After two months of painfully slow growth this indeed seemed to be the case. The isolated fungal colonies were now characterized by cells having large central vacuoles. In many instances the cells were abnormally swollen and were disintegrating. There was no evidence of fresh hyphae or any continuing growth. In contrast to this most of the contact groups, which also contained many starved hyphae, were still forming new branches. At the end of five months, however, all growth had virtually stopped and the cultures were abandoned. Although *Lecanora dispersa* was an excellent lichen to work with because of the proximity of its natural habitat to the laboratory, it had a big disadvantage in that the association of the fungal and algal symbionts was of the haustorial type. At no time was the presence of haustorial structures noted in the synthesis attempts. Perhaps a different lichen of similar habitat with a looser association of the symbionts, say of the appressorial type, would be more amenable to artificial synthesis in the laboratory. In these cases, the fungus could derive its nutriment from the alga by a simple diffusion after contact with the cell rather than by a direct penetration of the alga.

The algal symbiont on the agar film appeared healthy and continued to multiply by autospores and zoospores. Even after several months numerous free-swimming zoospores were noted after washing the culture; these flagellated spores rounded up as the culture film began to dry and some became enmeshed in the fungal hyphae. It seems quite probable, in my opinion, that the algae within a lichen thallus produce zoospores under moist conditions. This would be an excellent means of distributing the lichen over the same substratum. The rounded zoospores and lichen-fungal spores which are also released under the same moist conditions would thus form new colonies away from the parent plant.

Similar cultures on a fully nutritive medium (asparagine-glucose agar, pH 6.10) set up in the same manner and kept under the same conditions did not show these appressorial structures and both symbionts grew vigorously. The fungal hyphae, branching profusely, simply grew over the algal cells. Contacts which looked promising one day proved to be false the next as the hyphae grew beyond the cell. Hanging drop (Knop's solution) cultures also failed to give results, the fungus doing especially poorly in this case.

From the above observations it appears that the fungal component requires a solid substrate and has to be starved into this union. This seems to be in accord with the environmental habitat of the lichen.

In view of these results can it now be said that a true lichen synthesis has been effected? The answer to this question must be no. What has been achieved here is only a lichen by definition. The true, absolute synthesis must proceed from spore to spore via the obtaining of the lichen fruiting structures under controlled conditions.

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SURVEY OF SURFACE-ACTIVE CULTURE ADDITIVES FOR GROWTH OF PATHOGENIC HUMAN FUNGI [1]

R. M. Marwin

Surface-active agents, and in particular cationic agents, have been used for their depressing or inhibiting effect on the growth of microorganisms for a number of years. Glassman (**1948**) presented a comprehensive review of the literature describing surface-active agents and their application in bacteriology.

A lesser amount of research has been accomplished with the ability of certain surface-active agents, more frequently of the anionic type, to stimulate bacterial metabolism at low concentrations (Baker **1941**, Dubos **1947**). So far as the author has been able to determine, there have been no studies of the ability of these compounds to enhance the growth of fungi.

The present study was undertaken to determine if any of a large group of surface-active compounds were capable of stimulating the growth of a number of genera and species of human pathogenic fungi in a manner similar to that observed with other microorganisms. Progress on this problem was reported by the author in a preliminary abstract (Marwin **1956**).

MATERIALS AND METHODS

The 115 surface-active agents selected and surveyed in this study are representative of all three types, and together with their common names, their manufacturer, and type of activity when known, are listed in Table I. Considerably more than the 115 compounds were tested, but are not recorded here, as their solubility was not sufficient for these experiments. Currently there are well over 1000 surface-active agents offered on the market. While it must be recognized that the surface-active agents were manufactured for a wide range of industrial purposes where purity of product was not of primary concern (as it is for bio-

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TABLE I

SURFACE-ACTIVE AGENTS TESTED

Manufacturer	Trade Name	Type
Amalgamated Chemical Corp.	Dynesol R-50	Anionic
	Liquid Alkamine C Conc. #1	Non-ionic
	Liquid Alkamine C Conc. #2	Non-ionic
	Alkagel	Anionic
	Alkamine K-50	Anionic
	Alkamine D	Anionic
	Nionine LF	Non-ionic
Arnold Hoffman Co.	EC-203	Non-ionic
	DS-289	
	Ahcowet ANS	Anionic
	Ahcowet N	Non-ionic
	Synthrapol N	Non-ionic
Atlas Chemical Corp.	BRIJ 35	Non-ionic
	BRIJ 30	Non-ionic
	Renex 20	Non-ionic
	Renex 30	Non-ionic
	G-1690	Non-ionic
CIBA	Tergavone C	Anionic
	Solvadine G	Anionic
	Ultravon J. F.	Non-ionic
Colgate-Palmolive-Peet Co.	Low Foam Detergent	
	Arctic Syntex HD (Fab)	Anionic
	Ben Hur	Anionic
	Arctic Syntex M (Vel)	Anionic
	Halo	Anionic
	Ajax	Anionic
Dupont	Duponol PC	Anionic
	Duponol C	Anionic
Emulsol Chem. Corp.	Emcol E-607	Cationic
	Emcol 4150	Anionic
	Emcol E-11	Cationic
	Emcol 5100	Non-ionic
Fels & Co.	Felso	Anionic
Geigy Industrial Chemicals	Sarkosyl NL-100	Anionic
	Quaternary O	Cationic
	Sorbit AC	Anionic
	Nonisol 100	Non-ionic
Glyco Products Co., Inc.	Polyethylene Glycol 400, monolaurate	Non-ionic
Hart Products Corp.	Hartofol C	Anionic
	Hartopon B	Anionic
	Tergenol G	Anionic
	Hymolon K	Non-ionic
	Procol PW	

TABLE I—*Continued*

Manufacturer	Trade Name	Type
E. F. Houghton & Co.	Surfax WO	Anionic
	Surfax 505	Non-ionic
	Surfax 1288	Anionic
	Cerfak N-100	Non-ionic
	Cerfak 455	Amphoteric
	Cerfak 1300	Non-ionic
	Cerfak 1400	Non-ionic
	Surfax 1410	Anionic
Kessler Chemical Co.	Polyethylene Glycol 1000, monolaurate	Non-ionic
Lever Bros.	All	Non-ionic
	Breeze	Anionic
	Lux	
	Surf	Anionic
Michel & Co.	Michelene DCA	Anionic
Miranol Chem. Co., Inc.	Miranol C2M	Amphoteric
	Miracide LX	Cationic
	Miranol SM	Amphoteric
	Miranol CM	Amphoteric
	Miranol HM	Amphoteric
National Aniline Division	Nacconol Z	Anionic
	Nacconol NR	Anionic
	Nacconol SX	Anionic
	Nacconol DB	Anionic
	Nacconol NRSF	Anionic
Nopco Chemical Co.	Hyonic FA-20	Non-ionic
	Hyonic FA-75	Anionic
	Hyonic FA-40	Non-ionic
	Hyonic FS	Non-ionic
	Hyonic PE 225	Non-ionic
	Hyonic PE 250	Non-ionic
	Hyonic PE 275	Non-ionic
Ninol Laboratories, Inc.	Ninol 2012E	Non-ionic
	Ninex 300	Anionic
Onyx Oil & Chemical Co.	Super-Amide L9	Non-ionic
	Ammonyx TRM	Cationic
	Maprofix Powder LX	Anionic
	Isothan Q-15	Cationic
	Tetrosan 3, 4D	Cationic
	BTC 50%	Cationic
	Neutronyx 600	Non-ionic
Procter and Gamble	Cheer	Anionic
	Drene	Anionic
	Joy	
	Prell	Anionic
	Tide	Anionic
	Oxydol	Anionic

TABLE I—*Continued*

Manufacturer	Trade Name	Type
Quaker Chemical Products Corp.	Dianol F-50	
	Dianol G	Non-ionic
	Quaker X-1309	
Refined Products Corp.	Permalene A-100	Non-ionic
	PermaKleer 80	Anionic
Rohm & Haas Company	Triton X-400	Cationic
	Triton X-100	Non-ionic
	Triton 770	Anionic
Sandoz Chemical Works, Inc.	Sandopan DTC	Anionic
Synthetic Chemicals Inc.	Intracol O	Cationic
	Intramine 1080	Anionic
	Tensol N	Anionic
	Intramine Y	Anionic
	Intracol R	Cationic
	Negamine 142-A	Cationic
	Mulsor 224	Non-ionic
	Mulsor 3 CW	Non-ionic
Jacques Wolf & Co.	Oratol L-48	Anionic
	Parnol 40	Anionic
	Sellogen Conc.	Anionic
	Wetsit	Anionic
Wyandotte Chemicals Corp.	Kreelon 4 D	Anionic
	Pluronic L64	Non-ionic
	Pluronic L61	Non-ionic
	Pluronic L62	Non-ionic
	Pluronic F68	Non-ionic

logical research), it was felt that they were satisfactory for the initiation of a survey of this nature. As there was no way to determine initially the amount of each surfactant (surface-active agent) needed for growth experiments, it was necessary to utilize trial and error methods. The quantity of each diluted surfactant added to 100 ml lots of Sabouraud's dextrose agar adjusted to pH 6.8, which had previously been autoclaved and cooled to 45° C, was varied from 0.01 to 1.0 ml in increments of 0.01 ml. Those agents which were either too viscous, semi-solid, or solid were weighed in increasing aliquots of 0.01 gm, starting at 0.01 gm and increasing to 1.0 gm. This agar was poured into sterile Petri dishes in the amount of 20 ml a dish and surface inoculated according to the methods of Snyder (**1947**) with 0.1 ml aliquots from 10^{-5} dilutions of five-day Sabouraud's-dextrose-broth cultures of *Candida albicans* and *Cryptococcus neoformans*.

It was necessary to proceed with the inoculation of the filamentous

fungi by a different approach. One-half gram wet-weight lots of *Blastomyces dermatitidis, Microsporum Audouini, Epidermophyton floccosum, Sporotrichum Schenckii, Trichophyton mentagrophytes, Trichophyton rubrum,* and *Histoplasma capsulatum* were added to 100 ml of sterile saline and minced in a Waring tissue homogenizer. One-tenth ml aliquots of these organisms were then inoculated onto the same media according to the methods of Snyder.

Inocuiates of the yeast, yeast-like, and filamentous fungi were made onto the center of each plate of media and incubated at a controlled temperature of 23° C. Diameters of the colony growths of the yeasts and yeast-like organisms were recorded at 72, 96, and 120 hours. Similar readings of the slower growing filamentous organisms were recorded at 7, 10, 14, 17, 20, and 24 days. These numerous readings were necessary to check for variations in growth rates. Control plates of Sabouraud's dextrose agar without the surface-active additives were run simultaneously in a like manner. This procedure was selected because of its simplicity in screening a large number of compounds.

All organisms utilized were obtained from the American Type Culture Collection, Washington, D. C., and the departmental culture collection. Tests were replicated 20 times and averages of all growth diameters, and averages of minimum hours necessary to attain recognizable growth, were tabulated. An average of the largest and smallest diameters was taken when asymmetrical colonies were encountered. Microscopic examination *in situ* confirmed the recognition of organisms.

RESULTS AND DISCUSSION

The following four of the 115 surface-active agents screened were found definitely to augment the growth of nine human pathogenic fungi: Nonisol 100, Polyethylene glycol 400 monolaurate, Mulsor 224, and Pluronic L64. Average diameters of colony growth in mm on Sabouraud's agar with and without the addition of the surface-active agents are tabulated in TABLE II. The accelerating effect of these four surfactants is apparent when compared with the controls. The quantity of each surface-active agent which elicited the most favorable growth of the organisms when added to the basal medium is recorded with each product listed in TABLE II.

Colonial morphology of those organisms responding to the compounds tested indicated a slightly greater two-dimensional growth than that of the controls. Of even more significance was the third-dimensional aspect, particularly of the yeast-like organisms. Most of these colonies

were twice the height of the controls. Of the filamentous group, the aerial growth was not as pronounced as that of the yeast-like organisms. Their increased height was approximately only half that of the yeast-like fungi.

Amount of fungal growth and its measurement at selected time intervals has always presented several problems. Brown (**1923**) stated that even with its obvious drawbacks "the method of linear measurement is the best to apply in exploratory work." This method takes measurements along two marked directions at right angles to each other with an average computed of the two figures obtained. With a survey of this type involving thousands of measurements, this process permits

TABLE II

AVERAGE DIAMETERS OF COLONY GROWTH IN MM ON SABOURAUD'S AGAR WITH AND WITHOUT SURFACE-ACTIVE ADDITIVES

Organisms	Colony age in days	Sabouraud agar control	0.1 ml* Nonisol 100	0.3 ml* Poly. gly. mon. 400	0.2 ml* Mulsor 224	0.2 ml* Pluronic L64
Blastomyces dermatitidis	6	12.3	15.6	17.0	15.9	18.3
Candida albicans	2	3.8	6.2	5.8	7.0	7.1
Cryptococcus neoformans	2	7.0	10.3	11.3	12.6	10.4
Epidermophyton floccosum	6	6.4	10.7	11.2	9.6	11.3
Histoplasma capsulatum	8	3.9	6.3	5.9	6.8	7.1
Microsporum Audouini	8	9.1	14.7	13.8	14.0	12.7
Sporotrichum Schenckii	6	11.1	19.3	18.0	21.6	17.5
Trichophyton mentagrophytes	6	14.3	20.1	22.0	19.5	22.4
Trichophyton rubrum	6	13.7	19.9	21.5	23.2	25.8

* Per 100 ml sterile distilled water; one ml of each above dilution added to 100 ml agar.

replication and has the advantage of simplicity. Moreover, repeated measurements can be taken on the same colony as incubation continues. An objection raised to this method avers that no actual measure of the amount of mycelium produced can be ascertained, as linear growth may appear thin or dense. Continual stereoscopic observation of our organisms served to eliminate this contention.

Many problems of diagnostic clinical mycology revolve around the long incubation period required of fungi, and the additional hazard of bacterial contamination before fungal growth commences. It was found that the addition of these surfactants enabled the nine fungi studied to attain a recognizable stage of development in a shorter time interval than that shown on the control plates. These results are tabulated in TABLE III. Hours given are an average of the 20 trial runs.

All of the compounds which acclerated growth were water-soluble

members of the non-ionic group of surface-active agents. Nonisol 100 is a polyethylene glycol of lauric acid. A somewhat similar product, Polyethylene glycol 400 monolaurate, which also stimulated growth of the fungi, is a monoester of polyethylene glycol 400 and lauric acid. Mulsor 224 is a long-chain fatty-acid ester containing ether linkage, while Pluronic 164 is found to be a condensate of ethylene oxide with a hydrophobic base formed by condensing propylene oxide with propylene glycol.

TABLE III

AVERAGE OF MINIMUM HOURS REQUIRED TO ATTAIN DIAGNOSTIC GROWTH ON SABOURAUD'S AGAR WITH AND WITHOUT SURFACE-ACTIVE ADDITIVES

Organisms	Sabouraud agar control	0.1 ml* Nonisol 100	0.3 ml* Poly. gly. mon. 400	0.2 ml* Mulsor 224	0.2 ml* Pluronic L64
Blastomyces dermatitidis	123	92	89	85	81
Candida albicans	46	28	23	30	21
Cryptococcus neoformans	39	22	20	26	21
Epidermophyton floccosum	437	266	281	253	301
Histoplasma capsulatum	549	347	331	384	329
Microsporum Audouini	353	281	276	274	291
Sporotrichum Schenckii	178	130	141	109	107
Trichophyton mentagrophytes	332	246	251	229	263
Trichophyton rubrum	247	201	193	171	187

* Per 100 ml sterile distilled water; one ml of each above dilution added to 100 ml agar.

It is a well-known biological phenomenon that the addition of a wetting agent (such as the surfactants employed in this study) to media allows the nutrient material of the culture media to come in more intimate contact with the exterior of each growing cell and thus facilitates faster utilization of the nutrient with the consequent stimulation to growth of the organism (Frobisher **1957**). This principle has been applied to the isolation of the tubercle bacillus and several other microorganisms, but there is no evidence that it has ever before been investigated in relation to fungi.

SUMMARY

Following screening of 115 surface-active agents, four were selected for their abilities to enhance growth of human pathogenic fungi. It was found that 0.1 ml of Nonisol 100, 0.3 ml of Polyethylene glycol 400 monolaurate, 0.2 ml of Mulsor 224, and 0.2 ml of Pluronic L64 each incorporated separately, after dilution, into 100 ml of Sabouraud's dextrose agar would definitely accelerate growth of the following fungi:

Blastomyces dermatitidis, Microsporum Audouini, Candida albicans, Epidermophyton floccosum Sporotrichum Schenckii, Trichophyton mentagrophytes, Cryptococcus neoformans, Trichophyton rubrum, and *Histoplasma capsulatum.*

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A NEW MICROSPORUM AND ITS OCCURRENCE IN SOIL AND ON ANIMALS

LIBERO AJELLO

(WITH 12 FIGURES)

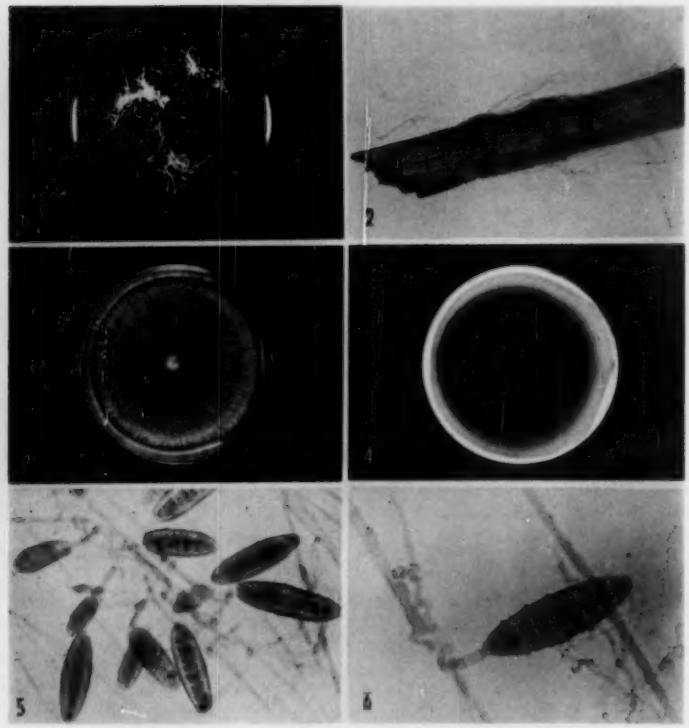
When plates of moistened soil are baited with sterilized strands of hair, according to a method developed by Vanbreuseghem (**21**), a large variety of keratinophilic fungi is obtained. Two of the commonest isolates recovered have proven to be the dermatophytes *Microsporum gypseum* and *Keratinomyces ajelloi* (**1**, **3**, **5**, **6**, **8**, **11–14**, **22**).

Among the other keratinophilic fungi found by this investigator, one is considered to merit recognition as a new species of *Microsporum*. It was first recovered in 1953 from a soil sample collected in a Saginaw, Michigan, barn. On the hair-bait it grew luxuriantly, covering the hair with whitish mycelium (FIG. 1). The fungus perforated the hair filaments (FIG. 2) and sporulated heavily. At this time it was noted that its macroconidia, although similar to those of *M. gypseum*, had thicker cell walls. Pure cultures of this mold were obtained readily with the use of cycloheximide agar (**10**).

The isolate grew rapidly on Sabouraud dextrose agar. Within a week with incubation at 25° C a mature colony had been formed. The colony had a yellowish central powdery area and a peripheral white downy zone. Through the downy area the purplish-red pigment that developed on the reverse of the colony was visible (FIGS. 3–4).

Microconidia as well as numerous macroconidia were produced. The multicellular macroconidia were elliptical and ranged from 45 to 75 μ in length and were 12–15 μ wide. Their echinulate cell walls were 2 to 5 μ in thickness (FIGS. 5–6). Pleomorphic changes occurred within three to four weeks.

The general morphology and method of sporulation of this fungus indicate that it is a member of the genus *Microsporum*. The unicellular microconidia and echinulate macroconidia borne on simple conidiophores are diagnostic of the genus. However, the development of elliptical macroconidia with thick walls and the consistent production of a purplish-red pigment on the reverse of the colony are criteria that serve to differentiate this fungus from all previously described and accepted species of *Microsporum* (TABLE 1).



FIGS. 1–6. *Microsporum cookei.* 1. Original Michigan soil plate with hair bait overgrown by *M. cookei.* 2. Perforations in hair invaded by *M. cookei.* 3. Mature colony of *M. cookei* on Sabouraud dextrose agar. Surface downy and pink. Michigan isolate. 4. Red-pigmented reverse side of above colony. 5. Group of *M. cookei* macroconidia from Sabouraud-dextrose-agar slide culture. Original magnification 470×. 6. Thick-walled, rough-surfaced macroconidum attached to conidiophore. Original magnification 970×.

After this organism was recovered from the Michigan soil sample, a large-scale animal-ringworm survey was initiated by the Mycology Unit of the Communicable Disease Center (**19**). In the course of this work, hair plucked from many of the wild mammals trapped in southwestern Georgia and northwestern Florida yielded this new fungus (**16–18, 20**). It was isolated from 219 animals representing 12 species (TABLE 2). It is significant that the host animals were free of obvious signs of infection. The hairs that yielded cultures did not fluoresce

when irradiated with ultra violet rays of 3660 Å. Many attempts to induce infections on laboratory mice, rabbits and guinea pigs as well as on my person ended in failure.

Recently Dr. William Kaplan of this laboratory recovered this organism from a dog in Pennsylvania that had ringworm lesions. Microscopic study of clinical material from the dog failed to reveal fungus elements although hair and skin scrapings yielded cultures of the fungus.

To date this laboratory has obtained 221 isolates of the new *Microsporum*, 220 from animals and 1 from soil. I propose the name *M. cookei* for this interesting keratinophilic mold in honor of the distinguished mycologist William Bridge Cooke.

Microsporum cookei sp. nov.

Colonia cum area centrali pulverea et lutea ad fuscidulam, zona peripherali tenui, alba, lanuginosa circumclusa. Color partis aversae obscure rubro-purpureus, per aream lanuginosam superficiei se manifestans. Mycelium hyalinum, 3–5 μ diam., saeptatum et ramosum. Microconidia hyalina et macroconidia parta in conidio-

TABLE 1

DIAGNOSTIC CHARACTERISTICS OF THE MICROSPORUMS

Species	Gross colony	Macroconidia
M. audouinii	Velvety, cream to tan, flat. Reverse with pinkish-brown center.	Absent or rare, numerous in rare isolates. Irregular to spindle-shaped, thick-walled, echinulate, few- to many-celled.
M. canis	Cottony, white to yellowish, flat. Reverse clear yellow or hyaline.	Abundant, spindle-shaped, thick-walled, echinulate, many-celled.
M. distortum (4, 15)	Velvety to fluffy, cream to tan. Reverse dull yellowish-tan.	Abundant, distorted, thick-walled, echinulate, many-celled.
M. gypseum	Powdery to granular, tan to cinnamon brown, flat. Reverse yellowish-tan.	Abundant, elliptical, thin-walled, echinulate, few-celled.
M. nanum (9)	Floccose and white, flat. Becoming granular and buff with tufts. Reverse at first orange later brownish-red.	Numerous, oval or elliptical thick-walled, smooth or echinulate. Non-septate or 1–2-septate.
M. cookei	Powdery with yellow-tan center, periphery downy white, flat. Reverse deep purplish-red.	Abundant, elliptical, thick-walled, echinulate, many-celled.

TABLE II

ANIMAL SPECIES YIELDING CULTURES OF MICROSPORUM COOKEI

Host species	Number positive	Number trapped	% Positive
Didelphis marsupialis (opossum)	22	874	2.5
Mephitis mephitis (striped skunk)	4	487	0.8
Mus musculus (house mouse)	3	525	0.6
Neotoma floridana (wood rat)	6	21	28.6
Peromyscus gossypinus (cotton rat)	33	111	29.7
P. nuttalli (golden mouse)	1	5	20.0
P. polionotus (old-field mouse)	23	584	3.9
Procyon lotor (raccoon)	2	1,177	0.2
Rattus norvegicus (Norway rat)	1	351	0.3
Reithrodontomys humulis (harvest mouse)	4	45	8.9
Sigmodon hispidus (cotton rat)	118	710	16.6
Sylvilagus floridanus (cottontail rabbit)	2	415	0.5
Grand Total	219	5,305	4.1%

With two exceptions all the animals yielding cultures were from the Georgia counties of Baker, Decatur, Dougherty, Grady, Mitchell and Thomas. One of the opossums and one of the striped skunks were from Leon County, Florida.

phoris simplicibus. Microconidia singillatim parta, clavata, 4 μ in longitudine, 2 μ in latitudine. Macroconidia numerosa, elliptica multisaeptata, parietibus cellarum 5 μ crassis in agaro farinae avenae, 2–5 μ in agaro dextroso Sabouraudi, superficie aculeata, 45–75 μ in longitudine, 15 μ in latitudine.[1]

Colony with a powdery, yellowish-tan [2] central area surrounded by a thin, downy, white peripheral zone. Reverse color deep purplish-red,[3] which shows through the surface downy area. Mycelium hyaline, 3–5 μ in diameter, septate and branched. Hyaline microconidia and macroconidia borne on simple conidiophores. Microconidia borne singly, clavate 3–8 μ long, 2 μ wide. Macroconidia numerous, elliptical, multiseptate, cell wall 5 μ thick on oatmeal agar,[4] 1–4 μ on Sabouraud dextrose agar, surface echinulate, 31–50 μ long, 10–15 μ wide on Sabouraud dextrose agar, 45–75 μ long and 15 μ wide on oatmeal agar.

Habitat: soil and various species of mammals. Known from Florida, Georgia, Idaho,[5] Michigan, Pennsylvania, and Washington,[5] U.S.A. and Alberta, Canada.[5] TYPE: Isolated from soil collected at Saginaw,

[1] I am indebted to Mr. Warren Laing Armstrong, CDC Technical Reports and Library Section, who prepared the Latin diagnosis.

[2] Munsell notation 10YR 8/6. Munsell Book of Color. Opposite Hue Edition. Munsell Color Co. Inc., Baltimore, Md. 1950.

[3] Ridgeway notation—Amaranth purple; Munsell equivalent 8.5 RP 3.2/10. Ridgeway, R. 1912. Color standards and color nomenclature. Washington, D. C.

[4] 5–10 fragments of oatmeal in 20 ml of 2% agar.

[5] See discussion.

Michigan, Aug. 7, 1953 (M30), and designated at the Communicable Disease Center as isolate B276.

DISCUSSION

After the recovery of *M. cookei* from Michigan soil, my attention was drawn to three isolates of "*M. gypseum*" isolated by Cooke (2). These isolates had been recovered from pieces of wool cloth buried in soil plots in the states of Washington and Idaho. Cloth buried in 3 of 18 plots yielded a fungus that produced a "deep blood-red pigment" on modified Sabouraud's agar. Abundant microconidia and macroconidia were produced. The ovate microconidia were 3–5 × 1.5–2–5 μ and the macroconidia measured 31–48 × 10–14 μ. "Warts" were scattered over the surface of the macroconidia. Their internal and external cell walls were up to 5 μ thick. Cooke was about to report his isolate as a new species but was advised against this by Emmons (7) who considered it to be a variant of *M. gypseum*.

Through the courtesy of Dr. C. G. Shaw, curator of the herbarium of Washington State College, Pullman, Washington, I was able to examine two of the wool-cloth pieces referred to above. Specimen #39107 was a 14.5 × 3 cm piece of wool charmeen that had been buried 1 cm deep in a soil plot in Idaho County, Idaho. Yellowish mycelial growth was present on several areas on the cloth. Some of this growth was scraped off, mounted in lactophenol-cotton-blue and examined under the microscope. Numerous macroconidia and some mycelium were observed among the debris. The spores were similar in size, form and cell-wall thickness to those produced by *M. cookei*. The second herbarium specimen, #39100, when examined proved to be free of fungus elements.

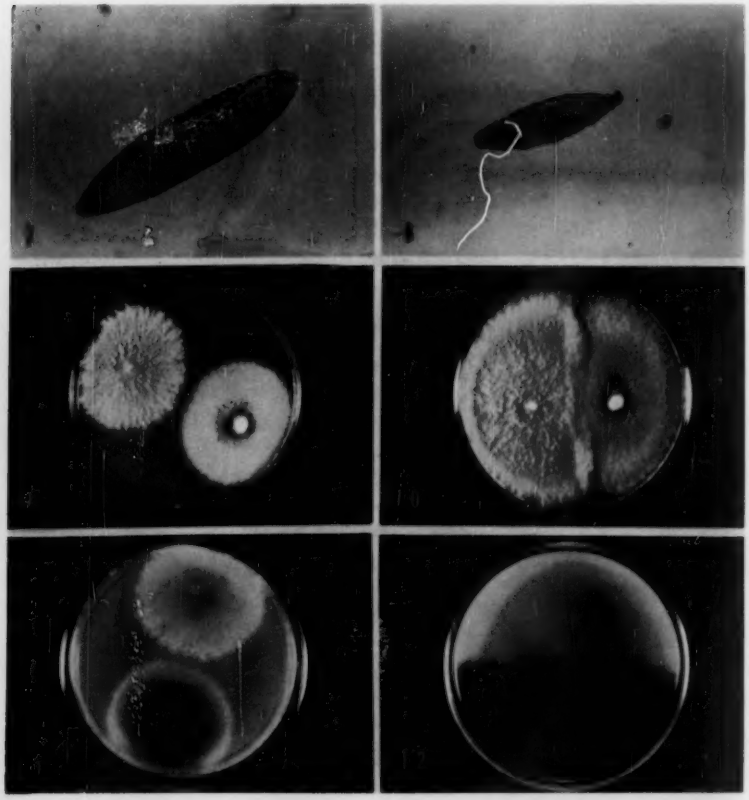
From Dr. C. W. Emmons a subculture of one of Cooke's isolates was obtained. This culture, number 246, had the gross and microscopic characteristics of *M. cookei*.

In my opinion *M. cookei* is not a variant of *M. gypseum*. The production of thick-walled macroconidia (FIGS. 7–8) and a deep purple-red pigment make it distinctly different from *M. gypseum* (FIGS. 9–12). Although pink or reddish variants of *M. gypseum* are encountered, their pigment is not similar to that of *M. cookei* and the macroconidial cell wall is never as thick.

On oatmeal agar after 2 weeks growth at 25°, the macroconidial cell wall of *M. gypseum* was found to be 1 μ in thickness; under similar conditions the cell wall thickness of *M. cookei* is 5 μ. When Sabouraud

dextrose agar is used as the growth medium the cell wall thickness in *M. gypseum* is also 1 μ but that of *M. cookei* averages 2.5 μ with a range of 1 to 5 μ.

Recently Dr. S. W. Carmichael of the Provincial Laboratory of



FIGS. 7–14. *Microsporum cookei*. 7. Macroconidium of *M. cookei* from an oatmeal-agar culture. Original magnification 970×. 8. Macroconidium of *M. gypseum* from an oatmeal-agar culture (Panama soil isolate, P50). Original magnification 970×. 9. Paired 9-day-old cultures of *M. gypseum* (upper left) and *M. cookei* on Sabouraud-dextrose agar. 10. Paired 26-day-old cultures of *M. gypseum* (left) and *M. cookei*. 11. Reverse of 9-day-old paired cultures of *M. gypseum* (upper colony) and *M. cookei*. 12. Reverse of 26-day-old cultures of *M. gypseum* (upper colony) and *M. cookei*.

Public Health, University of Alberta, Edmonton, Canada, forwarded a culture to this laboratory that he had isolated from soil through use of the hair-bait procedure. It proved to be *M. cookei*.

M. cookei appears to be widely distributed. It has been recovered from soil in the states of Idaho, Michigan and Washington and from Alberta, Canada. In addition it has been found to be associated with a large variety and number of wild animals in the states of Florida and Georgia and it has been recovered from a dog in Pennsylvania.

None of the carrier animals with the exception of the dog had lesions. Thus there is doubt concerning the pathogenicity of *M. cookei*. Although all attempts to induce experimental infections ended in failure, the possibility exists that, under certain circumstances, pathologic changes can occur. All of the wild mammals studied by this laboratory (**17**, **18**, **20**) from which the pathogenic dermatophytes *Trichophyton mentagrophytes*, *M. canis* and *M. gypseum* have been cultured also have been free of lesions.

Cultures of the Saginaw, Michigan, isolate of *M. cookei*, one of Cooke's isolates, and two from Georgia mammals have been deposited in the American Type Culture Collection, Washington, D. C., and the Centraal Bureau voor Schimmelcultures, Baarn, Netherlands.

Since this manuscript was submitted for publication, four additional isolates of *M. cookei* have been obtained: 1. Isolated from soil by Dr. Kåre Lindqvist, Veterinary College, Oslo, Norway. 2. Isolated from soil collected in Dalton, Georgia. 3 and 4. Isolated from two Persian kittens, which were free from lesions, by Dr. William Kaplan of this laboratory.

SUMMARY

A new species of *Microsporum* has been described. It differs from other species in the formation of elliptical, echinulate macroconidia that have a thick wall and in the production of a deep purplish-red pigment on the reverse of the colony.

M. cookei has been recovered from soil and from numerous animals.

COMMUNICABLE DISEASE CENTER
U. S. PUBLIC HEALTH SERVICE, DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
ATLANTA, GEORGIA

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COCHLIOBOLUS SATIVUS. II. PHOTO-ACTIVATED PIGMENTATION [1]

R. D. Tinline and D. J. Samborski

(with 7 figures)

Several pigments have been described from the mycelium of cultures of *Helminthosporium* spp. and some of them have been synthesized (**4**). Raistrick et al. (**6**) reported that species of the subgenus *Cylindro-Helminthosporium* of Nisikado (**3**) produced polyhydroxyanthraquinones whereas some members of the *Eu-Helminthosporium* subgenus produced a polyhydroxyxanthone. The imperfect state of *Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dastur, namely, *Helminthosporium sativum* P. K. & B. (*H. sorokinianum* Sacc. ex Sorokin), a member of the *Eu-Helminthosporium* group, frequently has been studied in laboratory cultures. However, there appears to be no record of pigment production by that species except for occasional references to the dark coloration of the spores and mycelium. An investigation of pigmentation in *C. sativus* was prompted by an observation of the senior author that a white-spored strain of the fungus when grown exposed to light produced a pink-colored mycelium; the mycelium was hyaline when light was excluded.

The present paper describes a pigment in *C. sativus* together with some aspects of its biosynthesis. For purposes of ready reference in this paper the pigment will be called sativin. The compound so named was not identified.

MATERIALS AND METHODS

Stock cultures of *Helminthosporium* spp. were maintained on potato-dextrose agar slants at 24° C. Monoascosporic and monoconidial dark- and monoascosporic white-spored strains of *C. sativus* were included in the series of cultures (**7**). Inocula consisted of aqueous suspensions of spores and hyphal fragments prepared from 7- to 14-day-old cultures, or 5-mm discs removed from actively growing colonies on potato-dextrose agar (PDA) in Petri dishes.

[1] Contribution No. 1702 from the Botany and Plant Pathology Division, Science Service, Department of Agriculture, Ottawa, Ontario.

A liquid basal medium (MM) was used for the production of still cultures. Its composition in grams per liter of distilled water was as follows: glucose, 10; $NaNO_3$, 3.12; $MgSO_4 \cdot 7H_2O$, 0.5; NaCl, 0.1; $CaCl_2$, 0.1; KH_2PO_4, 0.75; K_2HPO_4, 0.75; $ZnSO_4 \cdot 7H_2O$, 0.00021; $CuCl_2 \cdot 2H_2O$, 0.00005; MoO_3, 0.00005; $MnSO_4 \cdot 4H_2O$, 0.0027; $Na_2B_4O_7 \cdot 10H_2O$, 0.0025; and $FeC_6H_5O_7 \cdot 3H_2O$, 0.005. Potato dextrose agar generally was employed in the tests on solid media.

The media were of pH 5.8 to 6.8. Good growth of strains of *C. sativus* occurred within this range. Adjustments in pH to desired levels were obtained by the addition of 0.1, 0.2, and 1.0 N NaOH or HCl to the media after sterilization.

Cultures were grown at room temperature in the dark or exposed to sunlight. Light was excluded from the former cultures either by means of a light-proof box or by wrapping the culture vessels with aluminum foil. The influence of wave length of light on pigment formation was investigated by excluding all light except that which passed through Corning or Wratten glass filters.

The influence of nitrogen and carbon nutrition on growth and pigmentation was determined by culturing *C. sativus* on modified MM. In these modified media, the sodium nitrate was replaced by other nitrogen compounds in amounts in which the nitrogen added was equivalent to that provided by 3.12 g of $NaNO_3$ per liter. In a test in which the carbon source was varied the compounds replacing glucose were added in amounts which contained the carbon equivalent of 10 g of glucose per liter. The cultures were grown in chemically clean 250 ml Erlenmeyer flasks; each flask contained 50 ml of medium. The flasks were seeded with 1.0 ml of an aqueous spore suspension prepared from a white-spored strain. After incubation for 5 to 14 days at room temperature in sunlight, the mycelial mats were removed by the filtration of the cultures through a fine wire screen, oven-dried at 85° and weighed. Quantitative estimates of pigment formation were determined as follows: The pigment in a known weight of dry mycelium was diffusion extracted with acetone, the volume of acetone being directly proportional to the dry weight of the mycelium; the absorption of light by the centrifuged solution was the index of pigment quantity per milligram of dry mycelium. Light absorption was measured with a Beckman model DU spectrophotometer at a wave length of 518 or 520 mμ (millimicrons). Acetone solutions of the pigment could be diluted when necessary, since the dilution of the solution followed Beer's law. All tests were conducted in quadruplicate.

Absorption spectra of pigments in acetone solution from *Helmintho-*

sporium spp. were determined by the use of a Beckman model DU or Bausch & Lomb "Spectronic 20" spectrophotometer.

RESULTS

White- and dark-spored strains of *C. sativus* produced a pink pigment (sativin) in the mycelium when the strains were grown on PDA or MM and exposed to sunlight. The pigment failed to develop in duplicate series of cultures that were grown in the dark. In dark-spored cultures sativin was visible only in tufts of aerial mycelium and other zones which under dark conditions were composed of hyaline or light-colored mycelia. Generally the dark coloration of the spores and mycelium in the wild-type cultures visually masked the pigment.

Sativin was not uniformly distributed throughout the mycelium. Some hyphae were pigmented; others were not or only partially pigmented. The pigment appeared as a yellowish-orange color diffused through the cytoplasm with concentrated red areas, presumably in the vacuoles. Red granules were observed in the media among the hyphae.

The pigment was readily extracted with acetone or ether, less readily with petroleum ether or ethyl alcohol. The solvent was added to the agar cultures or to the dried mycelium from liquid cultures. Spores and other debris were removed from the solution by filtration and centrifugation.

Sativin formed in cultures exposed to sunlight regardless of the pH of the medium in which they were grown. In mycelium in acid media the pigment was pink; in highly alkaline media the pigment was blue; and in media of pH 7.1 to 8.5 the pigment was pink and blue. Absorption spectra of acetone solutions of pink and blue pigments from *H. carbonum* Ullstrup, and which appeared identical to those obtained from *C. sativus*, were similar (FIG. A).

Apparently the pigment is a pH indicator. Acetone solutions at pH values above and below the end-point of 8.6 were blue and pink respectively. The color change was reversible.

Observable pigment was formed in liquid cultures of a white-spored strain within 48 hours after the medium was seeded with spores. Cultures grown in MM in which casein hydrolysate was substituted for $NaNO_3$ and maintained in the dark for 5 days produced sativin in visible quantities within two hours of exposure to sunlight. Pigment concentration increased with time of light exposure throughout the six-hour duration of the test.

Sativin was produced more abundantly in young actively growing

cultures than in old declining ones (FIG. B). Flasks of MM were seeded at various times with an aqueous spore suspension held at 2° to prevent spore germination during storage. Cultures of the various ages grown in the dark at 24° were simultaneously exposed to sunlight for 7 days. Although maximum growth occurred at 15 days' incubation, maximum pigmentation occurred in the 9-day-old cultures. Sativin was not produced in the control cultures maintained for 7 and 39 days in the dark.

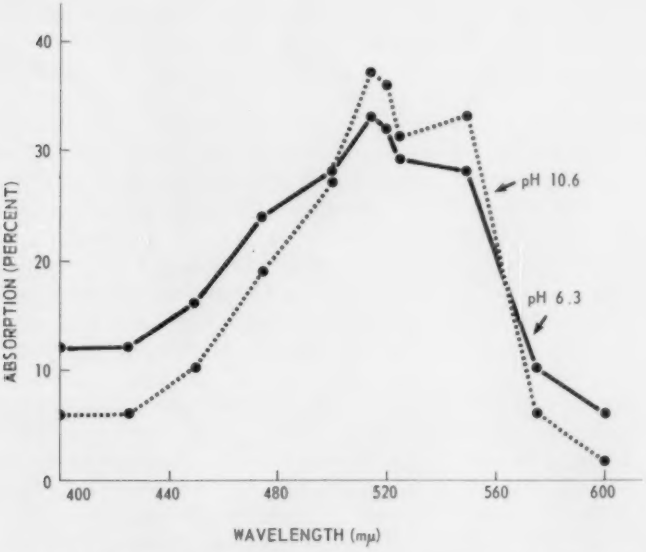


FIG. A. Absorption spectra of pink and blue pigments in acetone solution extracted from cultures of *Helminthosporium carbonum* on media of pH 6.3 and 10.6, respectively.

The quality of light influenced the production of sativin. Numerous tests were conducted in which filters were employed to control light wave length. The results of the tests were identical. The green, light-blue, and dark-blue filters under which pigment was formed (TABLE I) transmitted light from 460–580, 340–600, and 350–520 mμ, respectively; the red and yellow filters under which pigment was not produced (TABLE I) transmitted light from 580–760+ and 513–760+ mμ, respectively. Apparently light of the visible spectrum of wave lengths between 390 and

513 mμ, approximately, activated the biosynthesis of sativin. Ultraviolet and infrared light failed to promote the production of sativin as did light from tungsten lamps.

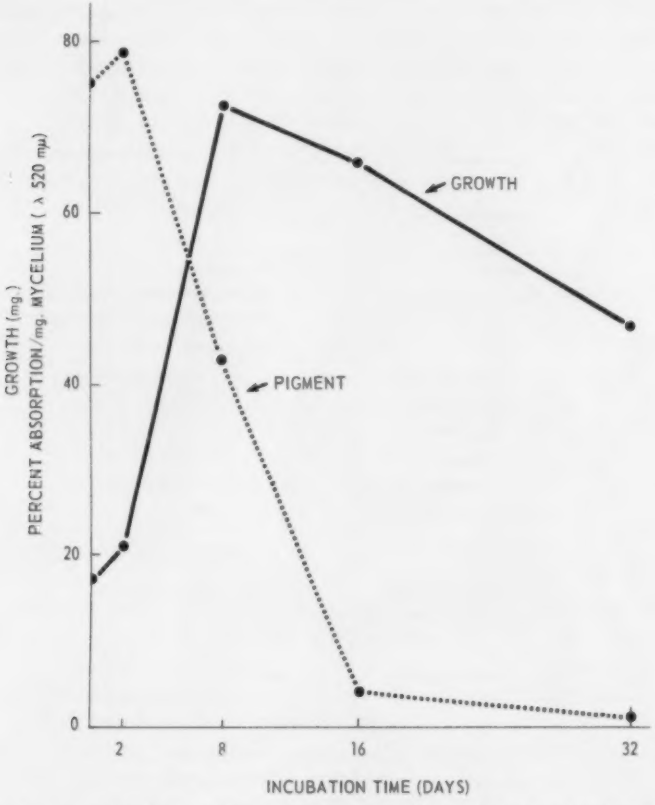


FIG. B. Growth and pigmentation of cultures of *Cochliobolus sativus* incubated for various periods in the dark, then exposed simultaneously to light for 7 days.

The production of sativin appears to be an oxidative process. One-week-old cultures of a white-spored strain on solid MM were placed in air-exchange desiccators and exposed to sunlight. Nitrogen that was freed of oxygen by passage over reduced copper was substituted for air in one desiccator. Upon incubation at room temperature for six days

sativin formed in the cultures under normal air atmosphere but not in those under a nitrogen atmosphere. The cultures from the latter treatment subsequently produced sativin upon removal to room atmosphere.

Pigmentation was precluded by some enzyme inhibitors but not by others. Mycelial mats from cultures of each of two white-spored strains, grown in the dark at 24° in MM of pH 5.4, were transferred aseptically to MM of the same pH that contained enzyme inhibitors. Upon incubation for three days at room temperature in sunlight sativin was observed in the cultures in the presence of dinitrophenol (10^{-5} M) and sodium fluoride (10^{-2} M). Sodium azide (10^{-2} M), potassium cyanide (10^{-2} M) and iodoacetate (10^{-2} M) inhibited pigment formation. In

TABLE I

EFFECT OF TRANSMITTED LIGHT ON PIGMENT FORMATION IN COCHLIOBOLUS SATIVUS GROWN ON MEDIA OF TWO pH LEVELS

Corning filter		Visible pigmentation in acetone solution*	
No.	Color	pH 6.3	pH 10.6
None (sunlight)		+++++	
None (dark)		−	−
2424	red	−	−
3486	yellow	−	−
4010	green	+	+
4503	light blue	++++	+++
5543	dark blue	+++	++

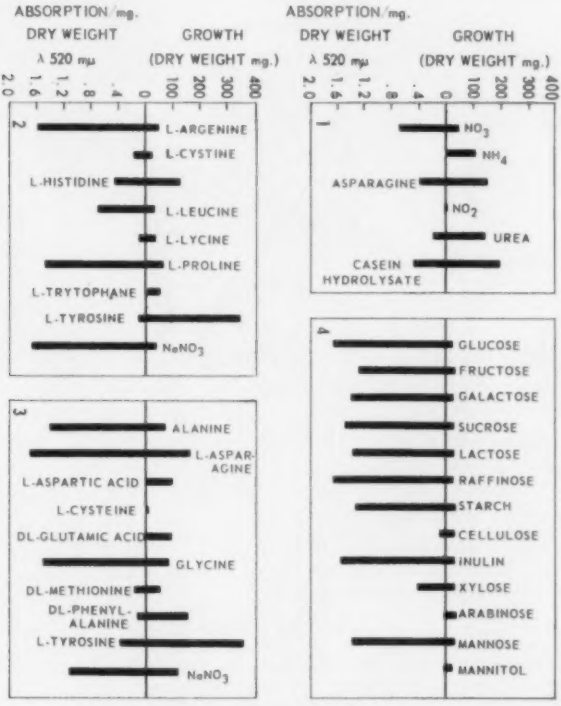
* The symbols ++, +, etc. indicate relative quantities of pigment; the symbol − indicates the absence of pigment.

other tests with sodium fluoride and dinitrophenol at concentrations of 10^{-2}, 4×10^{-2}, 8×10^{-2}, and 10^{-5}, 5×10^{-5}, 10^{-4} M, respectively, sativin was produced.

Considerable variability in sativin production was evident between and within strains of *C. sativus*. Strains which produced little or no pigment in one culture generation frequently produced abundant pigment in subsequent generations. Doubtless environmental conditions such as light, temperature, and nutrition affected pigmentation. Controlled tests of light and temperature were impractical. The influence of carbon and nitrogen nutrition on the formation of sativin, however, was investigated. The results of four tests are presented in FIGS. 1–4.

The nutrition that was provided the cultures greatly influenced growth and pigmentation as determined by dry weights and photoelectric measurements, respectively. Organic nitrogen supported more growth than inorganic nitrogen (FIG. 1). Ammonium nitrogen was

not favorable for pigment production. Pigmentation was not proportional to growth (FIGS. 1, 2, 3, 4). Some nitrogen compounds supported fungus growth yet failed to support pigment formation (FIGS. 1, 2, 3). Similarly, although growth of the fungus occurred to approxi-



FIGS. 1–4. Influence of nutrition on growth and pigmentation in *Cochliobolus sativus*. 1–3. Nitrogen sources. 4. Carbon sources.

mately the same amount on each carbon compound, pigmentation differed; on some compounds there was none, but on others it was abundant (FIG. 4).

Attempts to initiate formation of sativin *in vitro* and in dark-grown cultures were unsuccessful. Cell-free water and acetone extracts from light-grown colonies from which sativin had been removed were added to dark-grown cultures or to their water and acetone extracts. Pigment

formed only in dark-grown cultures which were subsequently exposed to sunlight.

Infrared spectra of partially purified samples of sativin and an anthraquinone were dissimilar. This suggests that sativin is not an anthraquinone.

TABLE II

COMPARISON OF ACETONE EXTRACTS FROM CULTURES OF HELMINTHOSPORIUM SPP. GROWN IN THE DARK AND EXPOSED TO SUNLIGHT

Species	Pigment			
	Peak absorption 490 mμ		Peak absorption 510 mμ	
	In light	In dark	In light	In dark
Subg. Eu-Helminthosporium				
H. carbonum	pink		pink	pink
H. cyclops	orange	orange		
H. halodes	orange		pink	
H. kusanoi				
H. leptochloae	orange		pink	pink
H. maydis			pink	
H. monoceras				
H. nodulosum			pink	
H. oryzae			pink	pink
H. portulacae				
H. ravenelii				
H. rostratum	orange	pink	pink	
H. sacchari				
H. sativum			pink	
H. setariae			pink	
H. stenospilum			pink	
H. victoriae			pink	
Subg. Cylindro-Helminthosporium				
H. avenae			pink	pink
H. dictyoides		orange*		
H. erythrospilum	orange			pink
H. gramineum	orange	orange		
H. teres	orange	orange		
Pyrenophora secalis	orange*	orange		

* Peak absorption occurred at a wavelength of 480 mμ.

Sativin was soluble in carbon tetrachloride, toluene, xylene, chloroform, ether, acetone, glacial acetic acid, and pyridine; moderately soluble in ethyl alcohol, n-butyl alcohol, and iso-propyl alcohol; insoluble in water, sodium hydroxide, and cold sodium carbonate solutions; soluble in boiling aqueous sodium carbonate. The pigment was pink in all solvents except aqueous sodium carbonate, in which it was blue.

Other *Helminthosporium* spp. produced pigments similar although

not necessarily identical to sativin. One or more isolates of various species were grown in MM or modified MM in which casein hydrolysate replaced $NaNO_3$ in light and dark conditions. Acetone extracts of wet or dried mycelia gave a visual indication of the presence or absence of

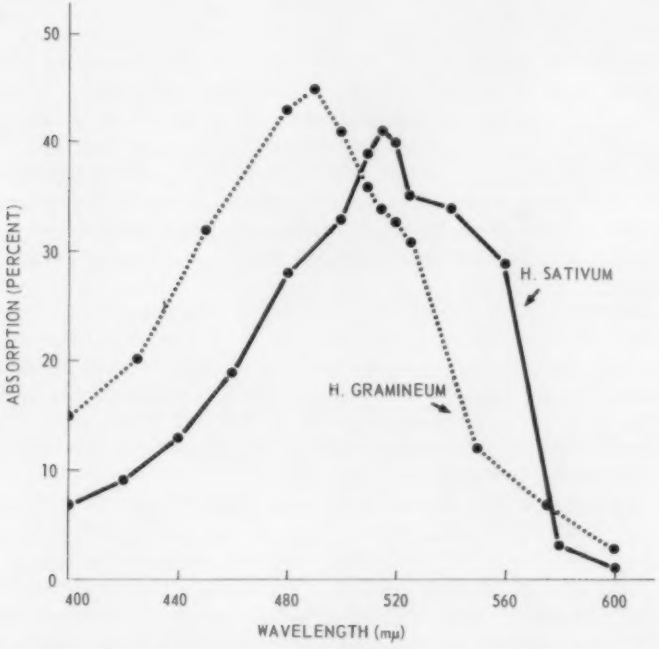


FIG. C. Absorption spectra of orange and pink pigments in acetone solution from cultures of *Helminthosporium gramineum* and *H. sativum* (*Cochliobolus sativus*), respectively, grown exposed to light.

pigments. Absorption spectra of the colored acetone solutions were determined after mycelial fragments and other debris were removed by centrifugation.

Possibly some species produced more than one pigment (TABLE II). An orange pigment with maximum light absorption generally at 490 mμ was extracted from some cultures; a pink pigment with peak absorption generally at 510 mμ was extracted from some of the same and different cultures. The difference in absorption spectra of the two colored solutions is illustrated in FIG. C. Several species synthesized pigment

regardless of light condition; in other species pigmentation appeared to be a photo-activated process as in *C. sativus;* still other species failed to produce detectable pigment.

DISCUSSION

Preliminary evidence suggests that the unidentified pigment which formed in the mycelium of *C. sativus* and which is referred to in the present paper by the name sativin is not an anthraquinone. Possibly it is a xanthone or a carotenoid compound. Raistrick et al. (**6**) observed that some species of the *Eu-Helminthosporium* subgenus to which the imperfect stage of *C. sativus* belongs produced polyhydroxyxanthones but not anthraquinones. Zalokar (**8**) confirmed that the formation of carotenoid pigments in *Neurospora crassa* was light-activated and of an oxidative nature. The production of sativin also required light and oxygen.

Although diffuse sunlight of wave lengths between 390 and 513 mμ induced pigmentation in *C. sativus,* light from tungsten lamps which encompassed these wave lengths failed to stimulate sativin formation. Presumably, the intensity of light from the latter sources was insufficient. In *N. crassa* illumination with a 100-watt white frosted light bulb at a distance of 20 cm for 1 minute was sufficient to stimulate full pigment production (**8**).

A number of *Helminthosporium* spp. produced pigments which appeared similar to sativin. Several species formed the colored compounds regardless of light condition. Further, some species apparently synthesized more than one pigment. The similarity of the pigments in appearance and in their absorption spectra in the visible light range would make difficult their use in species differentiation. Braverman (**1**), however, indicated that pigmentation and host range may be used successfully to distinguish species of *Helminthosporium.*

Considerable variability was observed within and between strains of *C. sativus* in the production of sativin. Probably the biosynthesis of the pigment is genetically controlled as is the synthesis of anthraquinones by *Cladosporium fulvum* (**2**). Spore-color mutants of the fungus are known; the colors are employed as markers in inheritance studies (unpublished data). Possibly mycelial pigmentation at such time as its formation can be standardized may also be used as a genetic marker.

Growth and the production of sativin varied with the age of the cultures and with nutrition. The pigmentation, however, was not proportional to growth. Peterson and Katznelson (**4**) reported that the

nitrogen source exerted a distinct effect on growth and the typical dark pigmentation in *H. sativum*. One strain studied produced excellent and moderate growth on media containing dl-B-phenylalanine and dl-asparagine, respectively. The mycelium produced on both nitrogen sources lacked the characteristic dark pigment. In the present work a white-spored strain utilized the nitrogen compounds almost equally. Sativin was formed more abundantly in mycelium on dl-asparagine than dl-B-phenylalanine. A comparison between tests of the growth response of the fungus to different nitrogen sources, however, is invalid. Different strains, media, and environmental conditions were used.

The inhibition of pigmentation by some enzyme inhibitors and not by others is interesting. Sodium azide and potassium cyanide inhibit terminal oxidases; the remaining inhibitors act on other systems. Since both sodium azide and potassium cyanide as well as iodoacetate prevented the formation of sativin there is some indication that terminal oxidases are involved in pigment formation. Possibly the production of sativin is dependent on a photo-activated process mediated by the terminal oxidases in the presence of oxygen.

SUMMARY

An unidentified pink pigment is reported from the mycelium of *Cochliobolus sativus*. Although present in all strains of the fungus that were tested it was generally detected visually only in white-spored lines. The pigment is water insoluble but soluble in organic solvents such as acetone, ether, and chloroform. It is a pH indicator with an end-point about 8.6, above and below which it is blue and pink, respectively.

Pigmentation was photo-activated. Light of wave lengths between 390 and 513 mμ stimulated the production of color. The light activation, however, was an oxidative process, since illumination in the absence of oxygen failed to promote pigment formation. The inhibition of pigmentation by potassium cyanide and sodium azide suggests that the process is mediated by terminal oxidases.

Pigment was produced in young actively growing cultures. Nutrition greatly influenced both growth and pigment production but not in proportionate amounts. Good growth of the fungus occurred on ammonium nitrogen but pigment was not formed; moderate growth occurred on nitrate nitrogen and abundant pigment was produced. Excellent growth developed on some organic nitrogen sources not all of which favored pigmentation. Although total growth of *C. sativus* was

similar on various carbon sources pigment production varied greatly. Media containing glucose and nitrogen as nitrate, alanine, l-asparagine, l-argenine, glycine, or l-proline appeared most conducive to both growth and pigmentation.

Other *Helminthosporium* spp. produced pigments which were similar in absorption spectra to that found in *C. sativus*. Color formation in several species, however, occurred regardless of light condition. Some species may synthesize more than one related pigment.

The authors are indebted to Miss Agnes Epp and Mr. G. B. Rennie of the National Research Council for conducting the infrared analyses and to Dr. M. Shaw, University of Saskatchewan, with whom the junior author was associated when part of this work was carried out.

CANADA AGRICULTURE RESEARCH LABORATORIES
SASKATOON, SASKATCHEWAN, AND
WINNIPEG, MANITOBA

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NOTES AND BRIEF ARTICLES

A System for Continual-flow Sea-water Cultures[1]

A fundamental problem among several, in the field of investigation dealing with marine fungi, is characterization of the rôle played by lignicolous fungi in the decomposition of wood and cordage submerged in sea water. Several methods of assaying this rôle are extant, but none yield more than indirect evidence. The point at issue is basically this: are lignicolous marine fungi the primary decomposers of wood submerged in sea water, or are they merely secondary invaders with decomposition being accomplished by some other group of organisms such as the ubiquitous marine bacteria? The mere growth of a lignicolous fungus in agar culture using various carbohydrates as the principal nutrition source does not reveal anything about the rôle of that fungus in the breakdown of wood in the natural marine or estuarine environment. Similarly, the fact that fungal hyphae penetrate submerged wood both inter- and intracellularly cannot be interpreted as conclusive evidence that fungi are the only primary decomposers of that wood. An assay method that employs the environment afforded by sea water, and that would, at the same time, eliminate the influence of any other organisms on submerged wood and cordage, is a necessity. A technique that affords continual circulation of raw, filter-sterilized, or autoclaved sea water over test wood and cordage samples inoculated with lignicolous marine fungi has been devised and put in operation as part of an exploratory study of the rôle of these fungi in the decomposition of submerged wood and cordage.

The circulating system (Fig. 1) is essentially a closed one, in which sea water is pumped continually over wood blocks (or rope pieces) that can be either inoculated directly with pure cultures of the test fungi or by the introduction of spore or conidial suspensions directly into the circulating water. The system may be either aerated or unaerated. Furthermore, the system can be sterilized and maintained in a sterile condition so that pure culture work can be accomplished.

The basic features of the circulating sea water system are the reser-

[1] The financial support of the National Science Foundation, through NSF Grant G-4016, is gratefully acknowledged.

voir (FIG. 1D), growth cylinders (FIG. 1G), a water pump, and an air pump. Pyrex carboys ($3\frac{1}{2}$ gallon capacity) are used as the reservoirs. These are modified standard carboys with two holes (for #9 rubber stoppers) drilled in the bottom. The carboy, either when filled or prior to filling, is inverted and supported by a tripod stand. The growth cylinders are $1\frac{3}{16}$ inch (i.d.) Pyrex glass tubes eight inches long. These are fitted with a suitable size rubber stopper, drilled with one hole, off-center. Six growth cylinders may be connected in series without reducing the water-pump efficiency or retarding inoculation of the en-

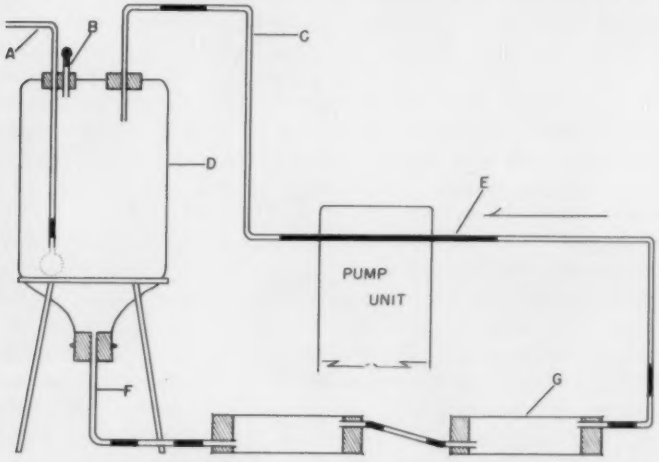

FIG. 1. Diagram of continual-flow sea-water system for submerged culture of lignicolous marine fungi. Direction of flow indicated by arrow; Tygon tubing sections are shown in solid black.

closed wood samples when inoculum is added directly to the water. The water is pumped through the cylinders and back into the reservoir by means of a variable speed Sigmamotor water pump and Zero-Max speed changer (Sigmamotor, Inc., Middleport, New York). Since the Sigmamotor pumps utilize a series of sequence-oscillating bars, the sea water does not come into contact with the metal of the pump. A Marco air pump (J. B. Maris Co., Bloomfield, New Jersey) with aerator head and line filter is used when aeration is desired.

Connections between the various parts of the circulating system are Tygon tubing (of a suitable diameter) and Pyrex glass tubing. Although all connecting lines except that running through the water-pump

housing can be made of glass tubing, a degree of flexibility is desirable because of vibration during pumping. For this reason, short sections of Tygon tubing are inserted into the glass-tubing connections at critical stress points. The short segments of Tygon at the inlet and outlet for each growth cylinder facilitate removal of the entire cylinder from the system when the wood blocks are to be harvested. The Tygon sections are closed with a pinchclamp, and the entire cylinder removed from the line. The water can then be drained from the cylinders and the wood blocks removed for weighing, examination, or sectioning.

If unaerated water reservoirs are used, two one-hole stoppers are fixed in the top holes of the inverted carboys. One stopper, with appropriate glass tubing, serves as the water return line (FIG. 1C), the second stopper, fitted with a short section of glass tubing closed at the upper end with a cotton plug, functions as an air release (FIG. 1B). Even in unaerated systems, the water pump cannot operate efficiently unless air pressures are automatically adjusted during pumping operation. When the reservoir water is to be aerated, the inverted carboy bottom is fitted with a one-hole and a two-hole rubber stopper (FIG. 1). The first stopper serves to hold the return line. The aerator line is affixed in one hole of the two-hole rubber stopper, and the second hole, with a short glass tube plugged with cotton, serves as the air pressure release. The one-hole rubber stopper in the inverted neck of the reservoir is wired to the flared mouth of the carboy, and fitted with glass tubing of a suitable diameter serving as the outlet (FIG. 1F) from the reservoir. By raising the reservoir above the level of the growth cylinders, gravity flow will fill the cylinders with water. Partial gravity flow obviously aids in pumping efficiency, and eliminates the need for a high capacity water pump.

Since the circulating system is bulky, sterilization of the unit must be done by sections. The reservoirs are sterilized by autoclaving the dry glass carboys for one-half hour at 250° F. The mouth and two bottom holes of the carboys are plugged with cotton prior to autoclaving. All glass connections that are positioned in rubber stoppers are sterilized by autoclaving them as units. The glass tubing is closed with small cotton plugs in each open end, the assembled tubing and stopper wrapped in paper, and the assembly sterilized. The longer pieces of glass tubing are also plugged at each end with cotton, and sterilized as separate units. Growth cylinders are first filled with the test wood or cordage, closed with the one-hole rubber stoppers (diametrically opposed), and the open ends of the inlet-outlet glass tubes plugged with cotton. These units are also wrapped and autoclaved. Sterilization of the Tygon tubing

pieces can be done either by a suitable germicide-fungicide solution or by autoclaving. The latter method is most suitable. Tygon of a slightly smaller diameter than the glass tubing is cut to the desired lengths, the ends are plugged with cotton, and the sections placed in deep Petri plates containing distilled water. The plates of submerged Tygon can then be autoclaved for 20 minutes at the usual sterilizing temperature without damage. The Tygon does become opalescent during the sterilization process, but after two or three days of use, the tubing regains its usual translucency. Some swelling of the Tygon sections occurs during autoclaving, but this is of no concern if the Tygon is initially of a smaller diameter than the glass tubing to which the sections are to be connected. Raw, filtered, aged sea water is autoclaved separately in 3-liter Fernbach flasks. The sea water can be sterilized in large capacity Pyrex separatory funnels that are subsequently attached temporarily to the inlet stopper of the reservoir and the water allowed to drain by gravity directly into the reservoir.

After all units are sterilized, the system may be assembled. Initial assembly consists merely of placing the rubber stoppers in the proper positions after removing any cotton plugs in the glass tubing. Subsequent assembly is accomplished by attaching the glass and Tygon tubing together, in their order of occurrence, always leaving the unattached end of any tube plugged with cotton until the next glass or Tygon section is attached. It is essential that the growth cylinders be assembled to the connecting lines in such a way that the inlet tube is below the outlet. This insures that the cylinders will fill with water and remain filled during operation of the system. When the growth cylinders and connecting lines are completely assembled, the reservoir is filled. The usual method of filling consists of fixing a sterilized Pyrex funnel to one of the holes in the inlet or air release stopper, and pouring the water directly into the reservoir.

Inoculation of the wood blocks or rope sections is done routinely by injecting a spore or conidial suspension of the fungus directly into the flowing water. This is accomplished by injecting the suspension with a sterile syringe and No. 26 needle into the small section of Tygon leading to the glass tubing in the reservoir inlet. The inoculum settles slowly to the bottom of the reservoir, and is subsequently circulated through the growth cylinders and remaining connecting lines by the water pump.

The dimensions of the wood blocks are not critical except for width. A block cut just slightly less than the inside diameter of the cylinder is most suitable. If the blocks fit loosely in the cylinder (through its

diameter) they swell sufficiently when wet to wedge firmly in the center of the cylinder, and will remain completely submerged. Routinely, six wood species are used in each cylinder.

The question of sterility in the filled and assembled system is naturally raised at this point. Even though the system must be assembled by sections, and the sea water poured directly into a temporarily opened reservoir, no bacterial or fungal contamination has been detected in any system even after three months operation. Testing for sterility is done by inserting a sterile hypodermic needle into any of the Tygon sections and drawing off a few milliliters of the water. The sample is then flooded onto a suitable sea-water or fresh-water medium, and the plate of agar subsequently observed for growth of bacteria or air-borne molds. A final test for sterility can be made when the wood or rope is "harvested" from the cylinders. Small shavings from the wood or rope are taken with a sterile scalpel and planted on agar; the plates are subsequently examined for contaminants. If contaminants are present in the system, they certainly do not appear by the sterility test methods employed.

Although the circulating water system does not permit exact duplication of the environment extant in natural sea water, it does afford a reasonable approximation. Certainly the growth of marine lignicolous fungi submerged in sea water in the system occurs under conditions that more nearly approach the environmental stresses of the natural habitat. The results of that growth can ostensibly be more reasonably interpreted as indicative of decomposition activities than pure-culture nutrition studies of the same fungi in agar culture. Two marine fungi have been tested extensively in the system. With both, the wood blocks show visible fungal growth within three days after the water is seeded. With both fungi, the extent of mycelial penetration into the submerged blocks has been determined by sectioning the wood radially, tangentially, and transversely. The depth of mycelial penetration by *Helicoma macrocephala* Kohlmeyer is far less than that of *Ceriosporopsis halima* Linder. Among other manifestations of decomposition, in the circulating sea water system, *H. macrocephala* induces a visible softening of the outer wood surfaces, whereas *C. halima* does not.

One important application of the continual-flow system is in facilitating a study of whether or not bacteria or fungi are the organisms principally involved in early stages of wood decomposition. By inoculating the submerged wood and cordage samples with various combinations and sequences of marine bacteria and fungi, one can note subsequent differences in breakdown of the cellulose material, at least in a qualitative

way.—T. W. JOHNSON, JR. AND HARVEY S. GOLD, Duke University, Durham, North Carolina.

AN UNDESCRIBED SPECIES OF PLATYGLOEA FROM IOWA

Platygloea abdita sp. nov. FIG. 1

Nullas fructificationes efformans, ut parasitus internus tantum occurrens in *Exidiopsis* fructificationibus; hyphis nodosis, 1.5–3 μ in diam.; basidiis cylindraceis, (2–)3-septatis, emergentibus, flexuosis, saepe omnino prostratis, (26–)30–45(–55) × 3–5 μ, typice basi plus minusve distincto probasidiali praeditis; epibasidiis 35 × 1.5–2 μ tenus; sporis ovatis, 5–7 × 3–3.5 μ, per repetitionem germinantibus; conidiis cylindraceis vel anguste clavatis, (4–)5–6(–8) × (2–)3(–3.5) μ.

Fructifications lacking, growing within the basidiocarps of *Exidiopsis ?sublilacina;* hyphae with clamps, 1.5–3 μ in diam., bearing conidiophores and basidia in the hymenial region of the host; basidia (26–)30–45(–55) μ in length, emergent from the host hymenium, usually bent at right angles and appressed, or, the metabasidium arising laterally from the probasidium and appressed; probasidia generally distinguishable, 8–10(–14) × 5–6 μ, functioning as the lowermost sporogenous cell; metabasidia cylindric, (2–)3-septate, 3–5 μ in diam.; epibasidia short or reaching up to 35 × 1.5–2 μ, arising at right angles to the metabasidium; basidiospores ovate, flattened adaxially, 5–7 × 3–3.5 μ, germinating by repetition; conidiophores complexly branched, the individual branches swollen to 2–4 μ in diam., the conidia borne singly and apically, each subtended by a clamp, the branches proliferating at maturity of the conidia and leaving the clamps as lateral projections, the tips of older branches with many such projections; conidia thin-walled, cylindric to narrowly clavate, the bases truncate or rounded, (4–)5–6(–8) × (2–)3(–3.5) μ.

Specimens examined: *R. J. B. 55–12,* TYPE, City Park, Iowa City, Ia., 9 Aug., 1955; *55–11,* 15 June, 1955, and *56–20,* 22 April, 1956, collected in the same locality as the type. All three were growing within fructifications of *Exidiopsis ?sublilacina* on wood of deciduous trees. All were collected in a low, poorly drained area adjacent to the Iowa River. Specimens are deposited in the herbaria of the State University of Iowa and the University of British Columbia.

This species is distinguished from *Platygloea peniophorae* Bourd. & Galz., and forms of that species lacking basidiocarps, by the presence of a distinct probasidium and by the structure of the conidiophores. *P. arrhytidiae* Olive is similar in that it lacks a basidiocarp and in basidial structure, but has different conidiophores and its hyphae lack clamps.

P. fungicola Olive has larger basidia, spores and conidia and is further distinguished in possessing paraphyses. The conidiophores of *P. abdita* are similar to those illustrated by Olive (Mycologia **39**: 92. *f. 3, d, e*) for a specimen found growing within *Dacrymyces* fructifications. However, no distinct probasidia were illustrated on the basidia of that fungus.

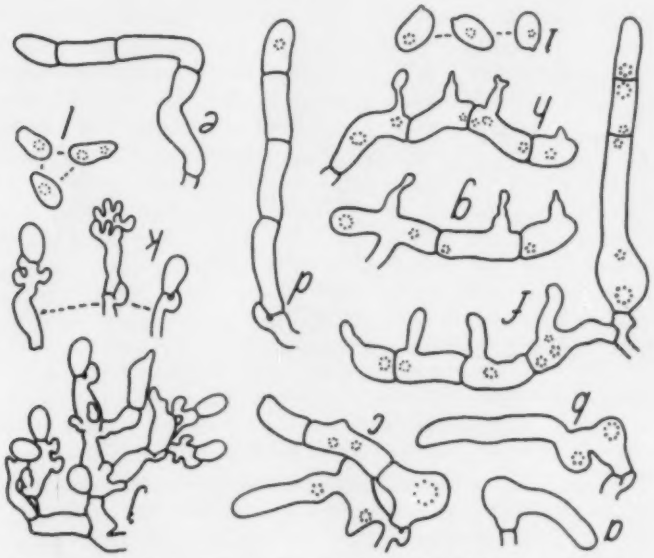

FIG. 1. *Platygloea abdita. a–h,* probasidia and basidia in various stages of development; *i,* three basidiospores; *j, k,* portions of conidiophores, some bearing terminal conidia; *l,* three conidia. (All figures drawn with the aid of a camera lucida and reduced in reproduction to approximately × 1000.)

The species mentioned above probably represent a group distinct from the remainder of the heterogeneous assortment of fungi now included in *Platygloea*. Their fungicolous habit, lack of basidiocarps, or, at most, possession of poorly developed basidiocarps which are mucedinous or weakly gelatinous, and constant production of conidiophores and conidia, sometimes to the near exclusion of basidia, distinguishes them from other species in the genus. Of the remaining species, only *P. miedzyrzecensis* Bres. and probably *P. disciformis* (Fries) Neuhoff are fungicolous and these are distinct in other respects. Although the latter has not been noted as being fungicolous, its fructifications are usually

associated with those of a pyrenomycete, sometimes producing basidia within the chambers of these fructifications.

Donk (Taxon **7**: 164. 1958) proposes the combination *Achroomyces disciformis* (Fries) Donk to replace *Platygloea disciformis* (Fries) Neuhoff. He feels that Bonorden's figures of *Achroomyces tumidus* (Handb. allg. Mykol. *pl. II, f. 231*) resemble structures of *P. disciformis* sufficiently well to warrant the assumption that they are identical, and he states: "Personally I would not suggest retention of *Platygloea* as a nomen conservandum." It is possible that Donk is correct in his assumptions, but I do not feel that Bonorden's figures resemble *P. disciformis* in the least. In my opinion, there can be no question of conservation of *Platygloea* over *Achroomyces* at the present time; such a question could only arise if concrete evidence, such as authentic specimens, were provided to show that *Achroomyces tumidus* Bonorden is identical with *Platygloea disciformis* (Fries) Neuhoff. Such evidence has not been presented as yet, hence the position of Bonorden's genus must remain doubtful while there are no such doubts concerning *Platygloea*.—ROBERT J. BANDONI, Dept. of Biology and Botany, The University of British Columbia, Vancouver 8, Canada.

ON CICINOBOLUS

The nature and biological relations of the common pycnidial parasite of powdery mildews have been well known since the publication of deBary's account of them in Beitr. Morph. Physiol. Pilze **3**: 53–75. 1870. The name generally applied to this fungus has been *Cicinnobolus Cesatii*, but there are difficulties in its continued employment.

As noted for the most part by deBary, the early synonymy for the parasite can be summarized as follows:

Ampelomyces Cesati ex Schlechtendal, Bot. Zeit. **10**: 302. 1852 (23 Apr.). TYPE: *A. quisqualis* Ces., Bot. Zeit. **10**: 301. 1852.

Cicinobolus [!] Ehrenberg, Bot. Zeit. **11**: 16. 1853 (7 Jan.). TYPE: *C. florentinus* Ehrenb., l.c. *Circinobolus* [!] apud Tulasne, Bot. Zeit. **11**: 265. 1853 (15 Apr.). *Circinnobolus* [!] apud Tul., Ann. Sci. Nat. Bot. IV. **6**: 309. 1856.

Byssocystis Riess, Hedwigia **1**: 23. 1853. TYPE: *B. textilis* Riess, Hedwigia **1**(5): 23. *pl. 3, f. 2, d–f.* 1853.

Cicinnobolus [!] *Cesatii* deBary, Abhandl. Senckenbirg. Naturf. Ges. **7**: 431 (Beitr. Morph. Phys. Pilze **3**: 71). *pl. 11; pl. 12, f. 5–13.* 1870.

The enumerated genera are monotypic, and the generic names and accompanying binomials are validly published, being accompanied by combined generic-specific descriptions (Art. 41). But they all have one fault: they all are, or may be considered, nomina confusa (Art. 66).

The pycnidial fungus was considered by Cesati, Ehrenberg, and Riess an autonomous parasite of the host plant, quite distinct from any erysiphaceous fungus; the mycelium and conidiophores of the latter were believed to pertain to the pycnidial fructification that in fact was only parasitic on them. Tulasne, on the other hand, in developing his central thesis of the pleomorphism of the fungi considered that not only the conidial fructification, the so-called *Oidium*, but likewise the pycnidia, pertained to the life-cycle of the ascomycete (Bot. Zeit. **11**: 265–267. 1853 (15 Apr.); C.-R. Acad. Sci. (Paris) **37**: 605–609. 1853 (post 17 Oct.); Ann. Sci. Nat. Bot. IV. **6**: 308–314. 1856; L.-R. & C. Tulasne, Sel. Fung. Carpol. **1**: 192–193, 216. *pl. 3, f. 2–14; pl. 4, f. 1; pl. 5, f. 1a, 2, 3.* 1861). Von Mohl (Bot. Zeit. **12**: 142–144. 1854 (3 Mar.)) reported both his confirmation of Tulasne's life-cycle and his observation of intergrading structures between the pycnidia and the cleistothecia developed on erysiphaceous mycelia. It was left to deBary to disentangle the mycelia and pycnidia of the parasite from the mycelia, conidial fructification, and ascocarps of the hosts and to set forth the descriptions and boundaries of the genera implicated.

DeBary's disentangling did not, unfortunately, extend to the nomenclature of the pycnidial fungus. He wrote (Beitr. Morph. Phys. Pilze **3**: 70, 71) that he was not obliged to retain any of the names earlier given to it, "da keiner derselben den Gegenstand bezeichnet, welchen ich zu benennen habe, sondern nur misskannte theile desselben," but wished to employ the "möglichst." Since to him the retention of Cesati's name and of Ehrenberg's binomial was "unmöglich," and to him Ehrenberg's generic name had priority, he chose to retain the latter, and to call the fungus in question *Cicinnobolus Cesatii*.

Such a procedure was wholly anomalous and cannot be followed under any rules of nomenclature, past or present. The descriptions of *Ampelomyces* (*quisqualis*), *Cicinobolus* (*florentinus*), and *Byssocystis* (*textilis*) cover essentially the same characters and embody the same errors. As Tulasne (1856) and deBary both learned, from authentic material of *A. quisqualis* and *B. textilis* and the description of *C. florentinus*, all these names are based on the same fungus. And of course priority belongs not to *Cicinobolus*, published in January, 1853, but to *Ampelomyces*, published in April, 1852. And finally, however, "impossible" the name *C. florentinus* may have seemed, it cannot be as impossi-

ble as retaining the name of that genus while rejecting the name of its type species.

Under the Rules there are two reasonable procedures. The first is to reject *Ampelomyces, Cicinobolus,* and *Byssocystis* and the names of their types, as "based on a type consisting of two or more entirely discordant elements." The second is to "select one of these elements as a satisfactory type" (Art. 66). If a name had ever been published for the pycnidial fungus alone, the first procedure, and the consequently legitimate name of the parasite, should of course be adopted. There seems to be none. Therefore it seems preferable, since the published names were all chiefly based on the pycnidia, to recognize these pycnidial fructifications and their own mycelia as the types of the names in question. In that case, the correct names for the fungus are the earliest, *Ampelomyces,* and *A. quisqualis.* As evidence that this does not do violence to the original concept, the original description is here offered, italics being supplied for those words referring not to the pycnidial parasite but to the erysiphaceous host:

"Ampelomyces (Nob.) sporang. minutiss., ochraceis v. lutescentibus, *brevi-pedicellatis,* subpyriformibus, torulosis, plerumque basi strumosa, *apice in tubam fere producto,* raro subdidymis (ut sporae Mycogones), sporidiis oblongis repletis."

In summary, the name *Cicinnobolus Cesatii* is wholly untenable for the pycnidial parasite of Erysiphaceae; it appears that *Ampelomyces quisqualis* Ces. can, and should, be employed.—D. P. ROGERS.

REVIEWS

BIBLIOGRAPHY ON THE EFFECTS OF IONIZING RADIATIONS ON PLANTS 1896–1955, by Arnold H. Sparrow, John P. Binnington, and Virginia Pond. v + 222 pp. Brookhaven National Laboratory, Upton, N. Y. 1958. Price $2.25; obtainable from the Office of Technical Services, Department of Commerce, Washington 25, D. C.

This extensive collection of citations includes about 2500 titles taken from nearly 500 journals and other publications. It has been published because, as the compilers state, "the botanical aspects of radiobiology were so extensive, so diffuse and so poorly covered by abstracting journals that few botanists, if any, could expect to be familiar with more than a small fraction of the existing publications." The subjects covered include most aspects of ionizing radiations on all groups of plants, with some explicit exceptions. The bibliography does not attempt to include tracer studies, nor studies made with ultraviolet radiations. Nor are bacteria considered.

The citations, arranged alphabetically by author, are given in the language they were published in (transliterated, if the original alphabet was not Roman), accompanied by an English translation of the title for each non-English work.

The subject index, which should be most useful, is made up from key words in the titles of the papers. Although it may as a consequence be limited in some cases by inadequately titled papers, this reviewer found that those topics he is interested in are satisfactorily represented in the index. Cross references are numerous. For example, A. E. Dimond's paper on the effects of continuous gamma radiation on tomato plants and *Fusarium* wilt is indexed under "fungi," "*Fusarium*," "gamma radiation, chronic," "tomato," and possibly others that I did not find. Further, it is suggested indirectly by such general references as "cobalt," "micro-organisms," "disease," etc.

Readers of this journal will be especially interested in the heading "fungi," under which nearly a hundred references are listed.

The senior author requests that investigators send him reprints and lists of pertinent references dealing with the years included in the bibliography as well as those since 1955.—DON RITCHIE.

NOMENCLATURE OF PLANTS, by Harold St. John. vii + 157 pp. Chronica Botanica New Series of Plant Science Books, No. 31. Ronald Press, New York, 1958. Price, $2.50.

This work bears the subtitle "A Text for the Application by the Case Method of the International Code of Botanical Nomenclature," and consists essentially of a large number (958) of citations of published taxa in various plant groups, designed to serve as material for independent nomenclatural solution by students as practice in interpreting nomenclatural problems in the light of the International Code. It is, in effect, a series of set exercises, grouped in chapters, with no solutions given, except in one sample case, that of *Cydonia Cydonia* (L.) Pers., which is worked out in detail in the Introduction, and which is shown to be an invalid name, synonymous with *Cydonia oblonga* Mill.

The set examples are chosen to bring the student, in his attempts to solve them, into contact with all the articles and recommendations of the Code of Botanical Nomenclature. A large number of cryptogamic taxa, including Fungi, figure among the set cases, most of them occurring in Chapter 3. Many of these naturally require extensive library facilities for their elucidation. The introductory part includes a listing of useful general publications (Indexes, Systems, Bibliographies, etc.) in various plant groups; under the heading of Fungi, only Saccardo's Sylloge Fungorum is mentioned, and here at least Clements & Shear's *Genera of Fungi* and Ainsworth & Bisby's *Dictionary of the Fungi* would have been helpful additions.

The reviewer feels that the usefulness of this book could have been increased by the addition of an appendix giving the correct solutions of all the exercises listed, with the step-by-step enumeration, by article and recommendation number, of the applicable provisions of the International Botanical Code in each case.—I. MACKENZIE LAMB.

BIOCHEMISTRY OF SOME PEPTIDE AND STEROID ANTIBIOTICS, by E. P. Abraham. vii + 96 pp., *7 figs.* John Wiley & Sons, New York, 1957. Price, $3.00.

This volume is the second of the CIBA lectures on microbial biochemistry to be published. The following subjects are reviewed: 1. the bacitracins, 2. the cephalosporins, and 3. structural and functional relationships of the bacitracins and cephalosporins. The author describes the methods used in separating and isolating these antibiotics and the

chemical studies which have revealed the main features of their structures. This information is the necessary basis for the last chapter, which is concerned with the relationship between structure and function of the bacitracins and cephalosporins and other related antibiotics. This book will be of value to those with a direct interest in antibiotics and their mode of action. It has values of another kind for those who are interested in physiologically active compounds found in natural materials, since it illustrates a way of attacking such complex problems. The author is Senior Research Officer at the Sir William Dunn School of Pathology, Oxford University. Dr. Abraham has long been active in the study of the chemistry of antibiotics; the present volume is based on a long and intimate study of the bacitracins and cephalosporins.—VIRGIL GREENE LILLY.

THE CLASSIFICATION OF LOWER ORGANISMS, by Herbert F. Copeland. ix + 302 pp., *45 figs.* Pacific Books, Palo Alto, 1956. Price, $7.50.

Few scientific disciplines have not, at one time or another, been bound by the fetters of tradition. The attempt to classify living organisms into a logical system which shall somehow approximate and reflect their relationship with others is perhaps by its very nature particularly susceptible to this danger. For this reason serious and informed attempts to rearrange systems in current use are to be welcomed and are entitled to careful and critical appraisal.

The aim, scope and contents of the book under consideration are admirably summarized in the first sentence of the introduction: "The purpose of this work is to persuade the community of biologists that the accepted primary classification of living things as two kingdoms, plants and animals, should be abandoned; that the kingdoms of plants and animals are to be given definite limits, and that the organisms excluded from them are to be organized as two other kingdoms."

The idea of more than two kingdoms is not new. It was vaguely suggested even before Haeckel proposed the kingdom Protista, and Copeland himself has for many years been advocating a system basically similar to that here developed in detail. The traditional view is still strongly intrenched, but there are at least a few indications that some biologists are sensitive to its inadequacies and are prepared to try to do something about it.

Copeland accepts Oparin's arguments that life originated but once

and that all living organisms are thus related. If this be true, then the four kingdoms are to be based on the acquisition of particular characteristics which, once attained, permit further advance. The most primitive organisms are presumed to have been without delimited nuclei: they are present today as the bacteria and blue-green algae, here united as Kingdom I, MYCHOTA. Nucleate organisms, not possessing the characters of plants or animals as defined, and including the fungi, the protozoa and the red and brown algae, comprise Kingdom II, PROTOCTISTA. Nucleate organisms with chloroplasts containing chlorophyll *a*, chlorophyll *b*, carotin and xanthophyll, and no other pigments, and which produce sucrose, true starch and true cellulose, including the green algae, the archegoniates and the vascular plants comprise Kingdom III, PLANTAE. Multicellular organisms with unwalled cells, which pass through blastula and gastrula stages in development and are typically predatory, are grouped in Kingdom IV, ANIMALIA. Only the first two kingdoms are discussed in detail.

The lack of a comprehensive nomenclatural code covering all organisms makes it impossible to follow any single code, hence the author has felt compelled to devise his own approach, discussed in Chapter II, an Essay on Nomenclature. The result is a large number of unfamiliar names and, on grammatical grounds, lack of uniform endings in names denoting groups of equivalent rank. The author does not regard this as important; to me it seems unfortunate. The usefulness of a uniform suffix denoting rank might well justify a certain freedom with the rules of grammar.

The PROTOCTISTA is divided into 12 phyla, the fungi in a restricted sense being included in 3 of them, with the Myxomycetes in a fourth. Phylum VI, the Phaeophyta, lists in Class 1, Heterokonta, as Order 5, the Hyphochytrialea, while Class 3, the Oomycetes, includes only the three orders Saprolegnina, Peronosporina and Lagenidialea. Phylum VIII, the Opisthoconta, includes the single Class Archimycetes, with the two orders Monoblepharidalea and Chytridinea. Phylum IX, the Inophyta, provides for the great bulk of the fungi, divided into four classes, the Zygomycetes, with 2 orders, the Ascomycetes, with 8 orders, the Hyphomycetes with 3 orders and the Basidiomycetes with 7 orders. Phylum X, the Protoplasta, Class 2, Mycetozoa, includes three orders, two for the Myxomycetes and one for the Phytomyxida (Plasmodiophorales). Certain smaller groups, commonly regarded as well established by mycologists, are ignored. For example, the family Tulasnellaceae is specifically suppressed as being "too small to be worthy of recognition." Yet the genus *Ceratiomyxa*, with, according to the author, "one well-

marked species," attains rank as a class, which, I hasten to add, it may deserve. The Class Hyphomycetes, for the three commonly recognized form orders of the imperfect fungi, is not, as the author admits, a phyletic unit, and it would seem that in a phyletic system some formal recognition should be made of that fact. Equally out of place in a phyletic system is the single order Dermatocarpea for all the gastromycetes.

Probably specialists in other groups could pick out similar flaws. But when all this is said, it remains true that in its extensive scope the work does present an over-all view of living things which can be defended logically and which brings into sharp focus the defects of earlier systems. The extensive bibliography (about 850 titles) represents sources that have been earnestly studied and carefully appraised. Regardless of the extent to which the author's views will be accepted, he has presented the biological public with some challenging views which should do much to stimulate thought in an area where it is needed.— G. W. MARTIN.

PRINCIPLES OF PLANT PATHOLOGY, by E. C. Stakman and J. George Harrar. xi + 581 pp., illus. Ronald Press, New York, 1957. Price, $8.00.

Plant pathologists for some time have realized that progress in the field has been impeded by the dominance of mycology in its practices during the period around the turn of the century. "Principles of plant pathology" puts the emphasis on the host-parasite-environment relationship rather than on the standard mycological approach to the subject. The table of contents emphasizes this approach: "The importance of plants," "the importance of plant diseases," "causes of plant diseases," "the production and liberation of inoculum," "the effect of environment and nutrition on disease development," "plant diseases of international importance," "international plant protection," "chemical control," "resistant varieties" and "future problems and prospects" are some of the chapter headings. The excellence and propriety of this approach are not to be questioned and when made by two of the more preeminent practioners in the field lead to great expectations. However, a careful reading of the text brings corresponding disappointment. The repetitiveness of the exposition, the too generous use of rhetorical questions, the over-emphasis on unpublished theses, the lack of pertinence of many of the otherwise excellent illustrations, the inadvertent omission of citations to im-

portant papers in the sections being discussed, all lead to the conclusion that sufficient care in the preparation of the manuscript was not given. It is unfortunate that a point of view of such potential importance should have been so cavalierly treated.

To gain acceptance of a new point of view in any field is very difficult under the best conditions; hence, it is regrettable that the excellent ideas and stimulating facts covered in this book did not have the careful handling that their importance warranted.—JOSEPH C. GILMAN.

FUNGOUS DISEASES AND THEIR TREATMENT, edited by R. W. Riddell and G. T. Stewart, with 34 contributors. xvii + 264 pp., *145 figures*, 54 tables. Butterworth & Co., London, 1958. Price, 45s.

This is a collection of 31 papers, with a foreword and an editors' preface, given at Brompton Hospital in a symposium sponsored by the British Postgraduate Medical Federation and supported by E. R. Squibb and Sons. The papers have been skillfully edited to maintain uniformity of style and to reduce repetition of material. The editors state in the preface that the symposium emphasized the need to reassess the pathogenicity of such fungi as *Candida albicans* and *Aspergillus fumigatus* and to look more carefully in England for systemic mycoses.

In a preliminary well illustrated chapter Riddell clearly summarizes the role of fungi as pathogens of man. Symmers, in a second well illustrated chapter, discusses the range of tissue reactions from the minimal response in cryptococcosis and mucormycosis to the suppurative reactions in blastomycosis, actinomycosis, nocardiosis and mycetomas. He mentions the advantages of polarized light to show birefringence and of periodic acid-Schiff, Gridley and silver stains to demonstrate fungi.

Vanbreuseghem reviews the dermatophytoses, the saprophytic occurrence of some dermatophytes and the relative importance of transmission of infection and of unknown factors influencing individual susceptibility and resistance. Calnan describes the clinical variations and increase in frequecy of *Trichophyton rubrum* infections; English reviews the epidemiology of *T. rubrum* infections, their familial occurrence and acquisition following foreign travel; and Tickner states that this fungus produces at least three pigments and that these are substituted anthraquinones. Holmes, reporting the frequency of tinea pedis in miners, concludes that exposure to the fungus is a more important factor in acquisition of infection than the degree of individual immunity.

Winner reviews the evidence indicating that the innate immunity of

animals varies with the species of the fungus and of the host animal, that an infecting exposure to be lethal must be above a critical threshold, that immunity can be experimentally induced and that natural immunity in man can be broken down by debilitating disease, administration of steroids or antibiotics, and by other conditions. Anderson reviews the occurrence of candidiasis following broad-spectrum antibiotic therapy; Donald reviews the clinical aspects and course of vulvo-vaginal candidiasis after antibacterial therapy; Dawkins, Edwards and Clayton report a semi-quantitative study of the presence of *Candida* during pregnancy with the characteristic postpartum decrease in this member of the vaginal flora; Bound describes cutaneous candidiasis in infants; Bodian reports 7 representative cases of candidiasis in infants and Cathie describes the pathology of this mycosis.

Mariat presents a good review based on extensive experience of mycetomas caused by species of *Actinomyces, Nocardia* and *Streptomyces* and describes the differential diagnostic features of the respective etiologic groups.

Both Hinson and Segretain discuss authoritatively the clinical forms and histopathology of aspergillosis. Citron and Pepys report clinical observations of aspergillosis patients with asthma and either with or without allergic sensitivity to aspergillin and without either of these manifestations of sensitivity. Fuller describes the clinical aspects of and possible bases for "farmers' lung," but without reaching a firm conclusion regarding any specific etiology.

Campbell presents an excellent review of the serodiagnosis of mycoses, compares technics and diagnostic antigens, reports results of extensive serodiagnostic surveys and discusses critically the occurrence and significance of cross reactions. Edwards reports extensive studies of the geographical distribution of histoplasmin sensitivity rates. Pierce describes the radiologic characteristics of various mycoses.

Stewart describes the *in vitro* effects upon morphology and growth of antifungals and the influence of alterations in the substrate (carbohydrates) upon activity of the drugs. Drouhet gives an excellent review of the chemistry, activity *in vitro*, therapeutic effect upon experimental mycoses and clinical use of each of several antifungal drugs and antibiotics. Barlow reviews the theoretical aspects of chemotherapy of skin infections and the chemistry and pharmacology of the commonly used drugs. Russell and Beare review standard methods of treatment of dermatophytoses. Jennison reports successful treatment of vaginal candidiasis with nystatin and amphotericin B, and Tubbs reviews the treatment of actinomycosis with penicillin. Plummer reports unsatisfactory

results in the chemotherapy of aspergillosis, presumably because of the inaccessibility of the fungus in bronchi or abscesses. Furcolow, in reviewing his therapeutic experiences in 60 cases of deep mycoses, reports favorable results in the treatment of cryptococcosis and histoplasmosis with amphotericin B.

In most of the chapters, at least in those dealing with therapy of the superficial fungus infections, recommendations are conservative, with reliance on the older drugs. In this respect, it is unfortunate that publication of the book preceded Gentles' epoch-making report of the excellent therapeutic effect in dermatophytosis of oral administration of griseofulvin.—C. W. EMMONS.

MANUSCRIPT

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